JOURNAL OF DAIRY SCIENCE

Contents

Studies on Ketosis in Dairy Cattle. I. Effect of Stall and Pasture Feeding upon the Concentration of Blood and Urinary Acetone Bodies of Dairy Cattle. C. B. KNODT, J. C. SHAW and G. C. WHITE 837

Studies on Ketosis in Dairy Cattle. II. Blood and Urinary Acetone Bodies of Dairy Cattle in Relation to Parturition, Lactation, Gestation, and Breed. C. B. KNODT, J. C. SHAW and G. C. WHITE 851

Studies on Ketosis in Dairy Cattle. III. Blood and Urinary Acetone Bodies as Related to Age. C. B. KNODT, J. C. SHAW and G. C. WHITE 861

Influence of pH, Type of Fat, and Pancreatic Extract upon Lipolysis in Homogenized Milk. I. A. GOULD 869

A Comparison of Rats Fed an Evaporated Milk with Those Fed a "Milk" in Which the Naturally Occurring Fat Has Been Replaced by Coconut Oil. SMITH FREEMAN and A. C. IVY 877

Bacteriological Study of Chocolate Milk. JAMES E. FULLER, W. S. MUELLER and R. W. SWANSON 883

The Effect of Stilbestrol and Anterior Pituitary Extract upon Lactation in Goats. A. A. LEWIS and C. W. TURNER 895

Abstracts of Literature A241

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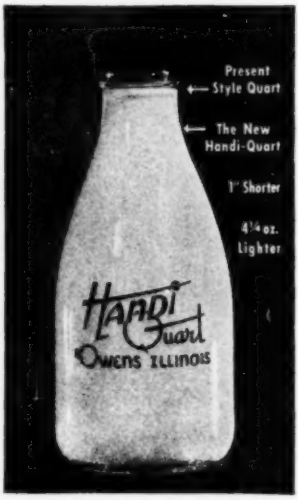

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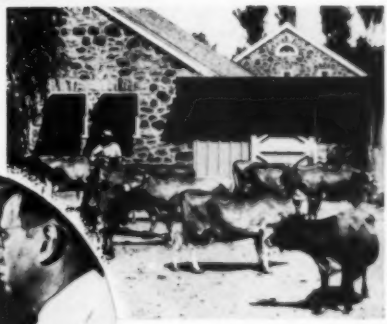

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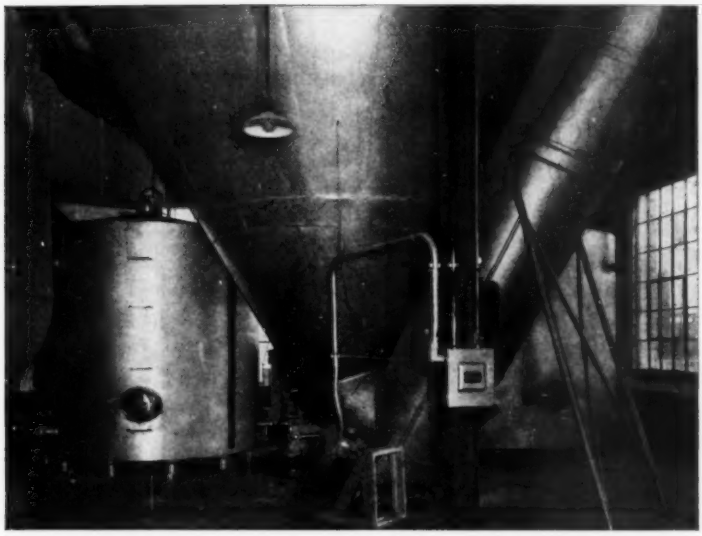

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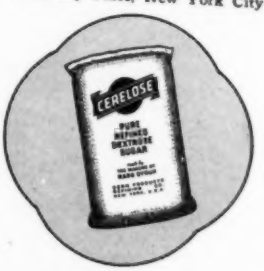

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JOURNAL OF DAIRY SCIENCE

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STUDIES ON KETOSIS IN DAIRY CATTLE. I. EFFECT OF STALL AND PASTURE FEEDING UPON THE CONCENTRATION OF BLOOD AND URINARY ACETONE BODIES OF DAIRY CATTLE[1]

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The problem of ketosis is becoming of as great concern to dairymen as it has been to professional workers during recent years. The fact that the incidence of ketosis is greatest in late winter and early spring and that it usually occurs shortly after parturition when there is an increased demand upon the body for nutrients for milk production has led many to believe that the causative factor or factors are nutritional. Others believe that the condition is pathological. This view receives support from the fact that it has been observed to be present in toxic conditions such as metritis, pleuritis, and hemorrhagic septicemia. While some of the aspects of the physiology of ketosis in cattle have been studied and several possible causative factors have been suggested, the actual predisposing factors which bring about "spontaneous" ketosis in cattle are not known.

In a review of the available literature, a total of 126 analyses for total blood acetone bodies of apparently normal cattle (3, 6, 7, 10, 16, 17, 19, 20) averaged 2.99 mg. per cent acetone (min. 0.69 and max. 5.54), while 58 blood acetone body analyses of cows diagnosed as having ketosis (3, 7, 8, 9, 11, 14, 16, 17, 18, 19, 20) averaged 33.61 mg. per cent acetone (min. 2.40 and max. 89.30). Eighty-six analyses (3, 4, 5, 7, 10, 16, 17, 19, 20) for urinary acetone bodies of apparently normal cows averaged 10.00 mg. per cent acetone (min. 2.50 and max. 70.00) and 53 analyses for urinary acetone bodies of cows diagnosed as having ketosis (3, 4, 7, 8, 9, 14, 15, 16, 17, 18, 19, 20, 22) averaged 253.77 mg. per cent acetone (min. 10.00 and max. 1568.00).

Various studies have been carried out in recent years pertaining to the blood and urinary acetone bodies in several species because of the importance of these substances in both normal and abnormal metabolism.

The literature in reference to ketosis in ruminants has been reviewed by

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[1] This project is financed in part by a grant from the Chas. M. Cox Co.

[2] The experimental data in this paper are taken from a thesis presented by C. B. Knodt in partial fulfillment of the requirements for the degree of Master of Science in Dairy Industry, University of Connecticut, Storrs Agricultural Experiment Station.

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Sampson and Hayden (18); Duncan, Huffman, and Tobin (7); Sampson and Boley (14) and others. For a review of the literature relating to the underlying mechanisms involved in the production of ketone bodies see Soskin and Levine (21).

It appeared that studies relative to the normal variations in the concentration of blood and urinary acetone bodies and their component fractions would give us some insight into the normal metabolism of ketone bodies in dairy cattle as well as some of the factors involved in the development of ketosis. Accordingly a rather comprehensive study was made of the normal variations in blood and urinary acetone bodies in the University dairy herd under the customary feeding regime.

EXPERIMENTAL

In the following experiments all blood samples were obtained from the jugular vein and potassium oxalate was used as the anticoagulant. The urine samples were obtained by massage of the vagina and were used only when a large amount of urine was excreted. All animals were apparently normal in every respect and care was taken to avoid excitation. The determinations were made immediately after the samples were obtained to minimize chemical changes which occur on standing. Blood and urinary acetone bodies were determined by the method of Barnes and Wick (1) and are expressed as mg. per cent acetone. β-hydroxybutyric acid was calculated by difference after determining the total acetone bodies and the fraction consisting of acetone and acetoacetic acid. Urine samples were diluted (1:1) with water before analyses to bring the concentration of acetone bodies closer to that of the blood acetone bodies and a corresponding correction was made in the calculation of the concentration of urinary acetone bodies.

Early in the course of our work considerable variation was observed in the concentration of the blood and urinary acetone bodies when consecutive samples were taken at short intervals during the day. As any considerable variation during the day in the level of the acetone bodies would be difficult to evaluate, an attempt was made to determine when the variations were greatest. The total blood and urinary acetone bodies were determined on samples drawn from two cows at intervals of two hours over a period of eight hours. In another experiment samples were drawn at two-hour intervals over a period of 24 hours and the fractions, acetone and acetoacetic acid, and β-hydroxybutyric acid were determined in addition to the total acetone bodies. In each of the three cases urine was drawn at 2:00 A.M. and discarded so that the samples drawn at 4:00 A.M. represented a two-hour excretion of urine. These cows received concentrates at 6:00 A.M., 2:00 P.M., and 10:00 P.M. Hay was fed daily at 10:30 P.M. and grass silage was fed twice daily at 8:15 A.M. and 2:30 P.M. The results are shown in table 1. Cows 1 and 2 showed an increase in total blood and urinary acetone bodies, which

TABLE 1

Variations in blood and urinary acetone bodies in mature cows at two-hour intervals (as mg. per cent acetone)

Cow no.	Time of day	Total blood acetone bodies	Total urinary acetone bodies
1	4:00 A.M.	3.40	13.72
	6:00 A.M.	3.15	9.37
	8:00 A.M.	4.21	12.61
	10:00 A.M.	5.02	19.97
	12:00 M.	5.63	20.80
2	4:00 A.M.	1.69	6.11
	6:00 A.M.	1.34	5.93
	8:00 A.M.	2.66	6.37
	10:00 A.M.	3.27	8.00
	12:00 M.	3.65	8.92

Cow no.	Time of day	Total acetone bodies		Acetone and acetoacetic acid		β-hydroxybutyric acid	
		Blood	Urine	Blood	Urine	Blood	Urine
3	2:00 A.M.	2.18	.....	0.36	.....	1.82	.....
	4:00 A.M.	2.46	17.74	0.61	6.14	1.85	11.60
	6:00 A.M.	2.19	15.66	0.18	5.28	2.01	10.38
	8:00 A.M.	2.25	16.52	0.63	4.96	1.62	11.56
	10:00 A.M.	2.37	15.08	0.67	5.10	1.70	9.98
	12:00 M.	3.82	17.50	0.82	4.80	3.00	12.70
	2:00 P.M.	2.87	10.80	0.48	3.32	2.39	7.48
	4:00 P.M.	1.46	11.90	0.72	2.62	0.74	9.28
	6:00 P.M.	2.55	17.54	0.42	5.66	2.13	11.88
	8:00 P.M.	2.33	18.00	0.51	6.84	1.82	11.16
	10:00 P.M.	2.30	11.48	0.61	5.04	1.69	6.44
	12:00 P.M.	2.14	12.32	0.30	4.28	1.84	8.04
	2:00 A.M.	2.41	16.10	0.23	4.68	2.18	11.42

was especially marked at 10:00 A.M. and 12:00 M. In view of the data presented in figure 3, to be discussed later, it appears that these marked increases were the result of the feeding of grass silage at 8:15 A.M. The smaller increases at 8:00 A.M. can logically be attributed to the feeding of concentrates at 6:00 A.M. on the basis of the same data. In the more detailed analyses in table 1 (Cow 3) the total blood and urinary acetone bodies were again high following the feeding of silage in the morning and were also high following the feeding of silage in the afternoon, although the effect was not as marked as in the other two cases. It will be observed that the larger variations in both blood and urinary total acetone bodies were usually due to variations in β-hydroxybutyric acid, the acetone and acetoacetic acid fraction usually accounting for only a small portion of the total change. Because of these observations all future samples were taken at 7:00 A.M. in an attempt to avoid the larger fluctuations which appeared to occur later in the day.

One series of studies included 16 purebred cows composed of six Holsteins, five Guernseys, three Ayrshires, and two Jerseys which were used for

the study of monthly variations in the concentration of total blood and urinary acetone bodies, acetone and acetoacetic acid, and β-hydroxybutyric acid. Five of these cows were dropped from the group during the 12-month period. Two were eliminated because of a tendency to become greatly excited during the drawing of the samples; one was sold from the herd; one calved prematurely and another was dropped from the group because of a severe edematous condition of the rear appendages prior to parturition.

The experimental period was begun on June 1, 1940, and samples were taken at monthly intervals to June 1, 1941.

All cows were on pasture daily from May 11, 1940, to October 12, 1940, and from May 2, 1941, to the completion of this work on June 1, 1941.

Three different commercial concentrate mixtures were fed but no variations in blood and urinary acetone bodies occurred which could be attributed to any of the three concentrate mixtures. These concentrate mixtures contained a large variety of grains and not less than 14 per cent crude protein.

Grass silage prepared by the addition of 80 pounds of molasses per ton of mixed grasses was the only silage fed. The cows received between 35 and 45 pounds of grass silage daily during the winter months, depending upon the weight of the animal. This amount was decreased by two-thirds when the cows went on early spring pasture and was gradually increased as summer progressed. Approximately the same amount of a fair quality of mixed grass hay, composed primarily of blue grass, was fed throughout the year.

The averages of the monthly analyses which were determined in a study of the normal variations in the concentration of blood and urinary acetone bodies are presented in figure 1. The initial samples were obtained in the month of June, 1940, after the cows had been on spring pasture for five to seven weeks. Both the blood and urinary acetone bodies were quite low at this time. There was an increase in the concentration of urinary acetone bodies and the component fractions, especially β-hydroxybutyric acid, as summer advanced. The concentration of blood acetone bodies was more variable, but a higher concentration of total blood acetone bodies was found during the months of September and October as compared to the month of June, 1940. A marked decrease in the concentration of blood and urinary acetone bodies and the component fractions was observed in November, 1940. A continual increase was observed in the concentration of blood and urinary acetone bodies beginning with the month of December, 1940, and continuing through March, 1941. Throughout the entire period of 12 months the increases and decreases in the total acetone bodies of blood and urine were accounted for primarily by changes in the concentration of β-hydroxybutyric acid. During the stall feeding period the rise in the fraction consisting of acetone and acetoacetic acid was also very marked in both blood and urine, although the increase was not as great as the increase in β-hydroxybutyric

acid. A large decrease occurred in May, 1941, in the concentration of total blood and urinary acetone bodies and of the component fractions two to three weeks after the cows had been on pasture.

The concentration of total blood and urinary acetone bodies of the cows decreased quite rapidly after the first day of pasture feeding as shown in table 2, with the former decreasing somewhat more rapidly on a percentage

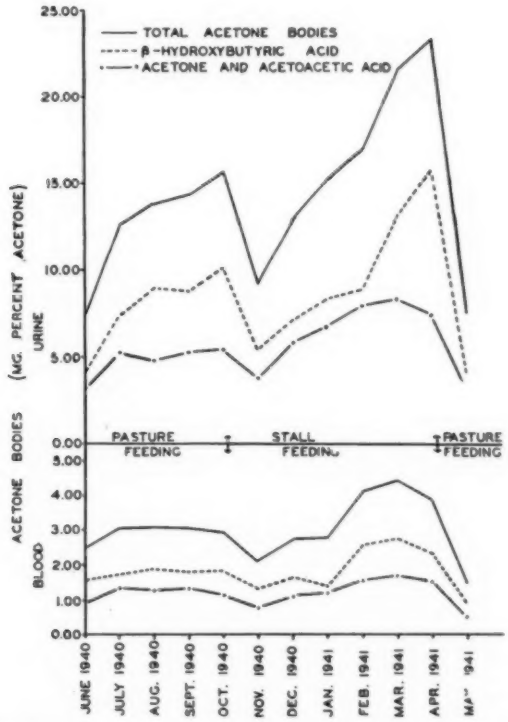

FIG. 1. Blood and urinary acetone bodies of mature cows on stall and pasture feeding. (Monthly analysis of 11 mature cows.)

basis than the latter. At the time the cows were placed on pasture the hay and grain feeding was continued at approximately the same level but the amount of grass silage fed was decreased by about two-thirds. It is of interest to note that the maximum decrease in acetone and acetoacetic acid in both blood and urine occurred after only one day of grass feeding. The blood and urinary β-hydroxybutyric acid on the other hand declined more gradually over a longer period of time, the decrease in total blood and urinary

acetone bodies after the first day of pasture feeding being due almost entirely to a decrease in β-hydroxybutyric acid.

Figure 2 presents the analyses for blood and urinary acetone bodies of a six-year-old Jersey bull at monthly intervals for a one-year period. In stall feeding the bull received a grain ration similar to that received by the herd cows in addition to approximately 10 pounds of grass silage per day and a

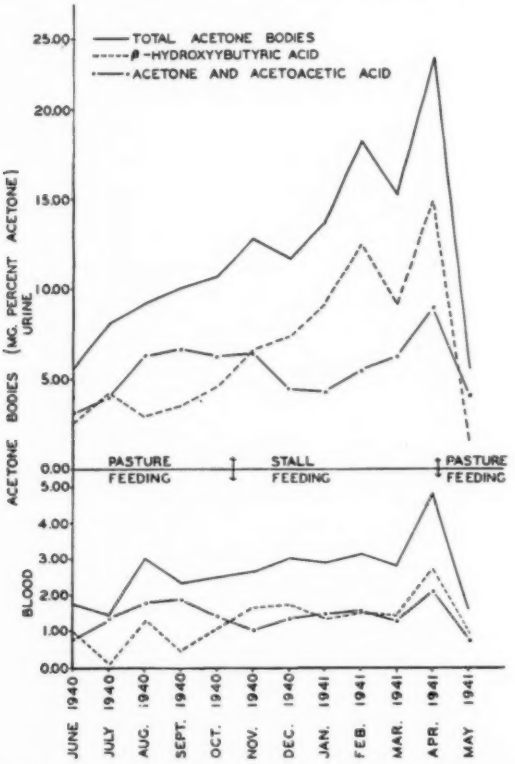

FIG. 2. Blood and urinary acetone bodies of a mature bull on stall and pasture feeding.

fair quality of mixed grass hay. The bull was on pasture daily in the first summer to October 12, 1940, and after April 26, 1941, to the end of the experimental period. The lowest concentrations of blood and urinary acetone bodies and component fractions were observed during spring and early summer pasture and the highest concentrations were found in the April, 1941, analyses after six months of stall feeding. These observations are quite similar to those made on the cows during this same period.

TABLE 2

Blood and urinary acetone bodies of 6 mature dairy cows on pasture feeding following 7 months of stall feeding

Date of analysis	Acetone bodies (expressed as mg. per cent acetone)					
	Acetone and acetoacetic acid		Total acetone bodies		β-hydroxybutyric acid	
	Blood	Urine	Blood	Urine	Blood	Urine
5– 2–41	0.98	4.70	2.71	11.10	1.73	6.40
5– 3–41	0.32	2.79	1.98	9.88	1.66	7.09
5– 4–41	0.48	3.13	1.59	8.91	1.11	5.78
5–10–41	0.51	3.10	1.60	7.92	1.09	4.82
5–18–41	0.43	2.55	1.28	5.38	0.85	2.83

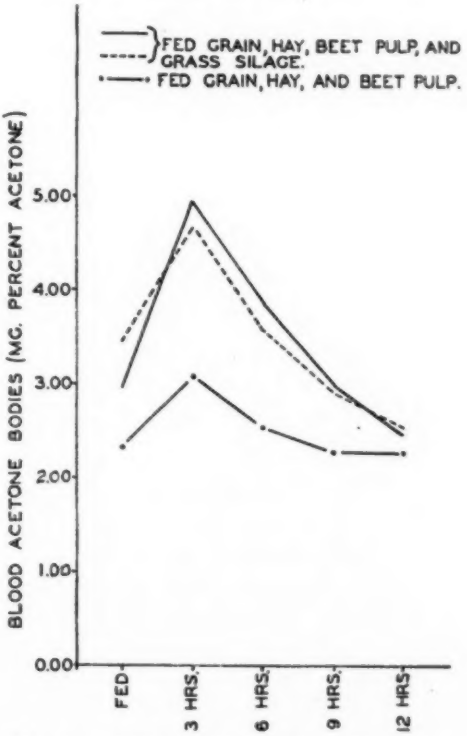

FIG. 3. Effect of grass silage feeding upon the blood acetone bodies of 3 mature cows.

To ascertain the immediate effect of silage feeding upon the concentration of blood acetone bodies, the experiment outlined below was conducted. Three cows which had not received any feed after 10:00 P.M. of the previous

evening were fed the following feeds between 5:30 A.M. and 6:30 A.M. on the following morning: two cows received grain, beet pulp, fair quality mixed grass hay, and approximately 15 pounds of grass silage; one cow received the same ration with the exclusion of grass silage. No feed was received thereafter by these animals for 12 hours and blood samples were drawn at three-

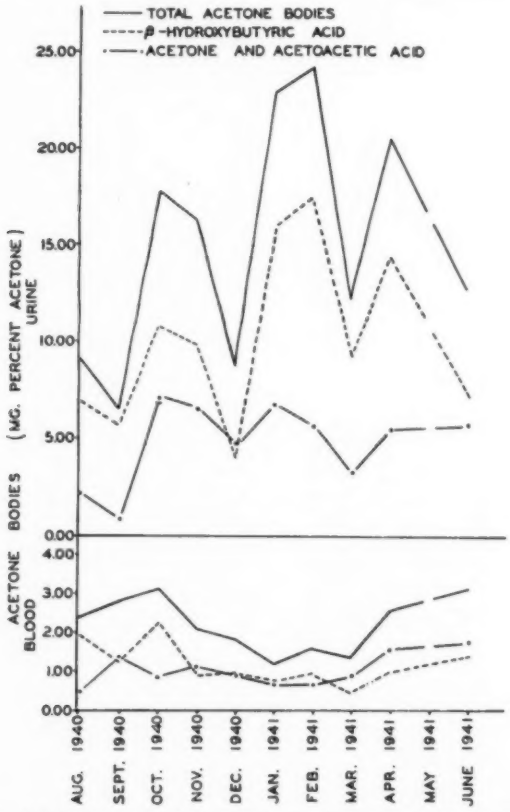

FIG. 4. Blood and urinary acetone bodies of 4 heifers 16 to 20 months of age as of August 1, 1940.

hour intervals beginning prior to the time of feeding and extending over a period of 12 hours. The variations in the concentration of blood acetone bodies over the 12-hour period are presented in figure 3. There was a rise in the acetone bodies of all three cows two hours after feeding followed by a rather marked decrease, approaching the previous level, after a period of nine hours. The cows receiving grass silage showed a much more marked increase in the blood acetone bodies, indicating that grass silage feeding

produced a much greater increase in the blood acetone bodies than grain and hay feeding alone. The effect of feeding in this experiment was more clear-cut than that shown in figure 1, probably because other feeds were not given during the day as in the previous experiment.

Data are presented in figure 4 on four heifers ranging from 16 to 20 months of age at the beginning of the experiment which extended essentially

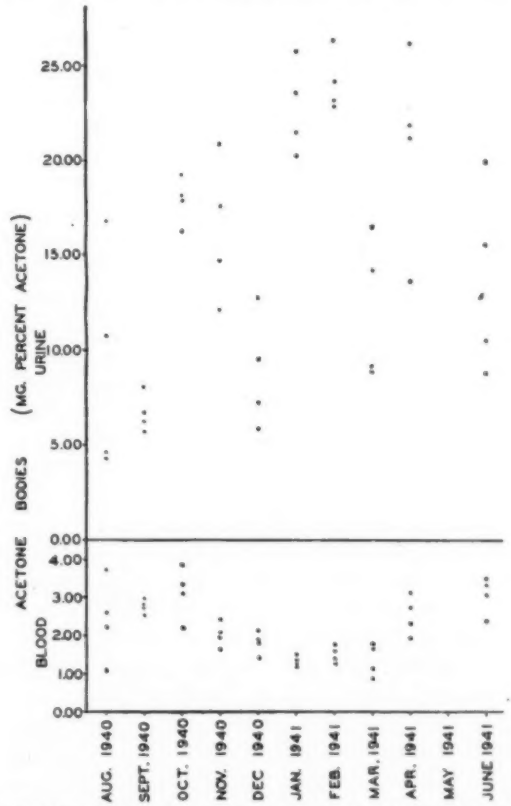

FIG. 5. Total blood and urinary acetone bodies of 4 heifers 16 to 20 months of age as of August 1, 1940.

over the same period as that of the cows and the mature bull. These heifers were in pasture lots the year around and did not receive any additional feed during the summer months. Very little grass was available between October and April, however, and during this period the ration consisted entirely of a fair quality of mixed grass hay. The urinary values were extremely variable and presented no definite trend. The acetone bodies in the blood indicated a trend in which, surprisingly, the low values were observed during

the winter period on hay feeding while the high values occurred during the grass feeding period. As was observed in the mature cows and the aged bull, the marked variations in the urinary acetone bodies were due primarily to β-hydroxybutyric acid.

The scatter diagram of the total blood and urinary acetone bodies of this group of heifers is presented in figure 5. While the urinary acetone bodies were extremely variable, the total blood acetone bodies showed much less dispersion.

DISCUSSION

The variations observed in the blood and urinary acetone bodies of the mature cows and aged bull appear to be rather closely associated with pasture and silage feeding. It has been observed by Brouwer and Dijkstra (4) that when large quantities of silage of the butyric acid type were fed there were marked increases in the urinary acetone bodies. McAuliffe, Stone and Bechdel (13) reported an increase in volatile acids over a period of time in alfalfa silage prepared by the addition of molasses. Bender, Bosshardt, and Garrett (2) reported an increase in acetic acid, from 0.12 to 1.18 per cent, over a period of 35 days following ensilation. Most of this increase was observed during the first 10 days. The largest increase in the blood and urinary acetone bodies occurred in the cows and aged bull on stall feeding when the animals were receiving silage in which similar fermentations had probably taken place. In the mature cows low values were observed in June of 1940 and May of 1941, as well as in November of 1940. These periods correspond to spring and early summer pasture feeding and the feeding of fresh silage made from third crop alfalfa hay respectively.

The increases all occurred after the silage was at least several weeks old. This is of particular interest since it has been shown by MacKay, *et al.* (12) that the feeding of acetic acid to a phlorhizinized dog and fasting rats increased the acetone body production. The marked decrease in May 1941, which occurred after the cows were on grass was undoubtedly due in part to the decrease in the feeding of grass silage at this time. However, the fact that the acetone bodies continued to decrease over a 16-day period to such low values indicates that grass may have had an additional effect beyond that produced by the lowered level of silage feeding.

That the increase in the acetone bodies of the cows during the stall feeding period was more closely associated with the feeding of grass silage than any other single factor is borne out by the data, in figures 4 and 5, on four heifers which had not received silage at any time during the year. The lowest values were observed on these heifers during the winter months when the ration consisted solely of fair quality hay. The principal difference between these two groups in the blood acetone bodies was the high concentration of β-hydroxybutyric acid in the cows during the period of aged grass silage feeding. It is, indeed, puzzling that the blood acetone bodies of the heifers

should have been so much lower on a ration of mixed grass hay than on pasture grass. The hay may have supplied less ketogenic material than the grass or the rumen flora may have been changed to such an extent that smaller quantities of acetone body precursors were formed in the rumen. This appears to be contrary to the data indicating that pasture grass produced a decline in the acetone bodies of the mature cows below that to be expected from the decline in silage intake.

As the level of acetone bodies in other species has been shown to be markedly affected by variations in energy intake this aspect must also be given consideration in any suggested explanation of the cause of the variations observed in these experiments. However, the variations observed do not appear to be associated with the relative energy intake. The milking cows which were rather heavily fed exhibited much greater concentrations of acetone bodies than the heifers which were maintained at a rather low nutritive level on mixed grass hay. This is further borne out by the data on the bull which was fed at a higher nutritive level than the heifers. The fact that the blood acetone bodies of the heifers were higher when the animals were on grass than on hay also suggests that rumen digestion may play an important part in the production of acetone bodies in the bovine.

The acetone and acetoacetic acid is relatively much more constant than the β-hydroxybutyric acid. This may indicate that ketone bodies are formed either directly or indirectly as the result of digestion in the rumen in which β-hydroxybutyric acid is the chief component. At least a part of the ketone bodies in the blood and urine of the bovine may be expected to have an endogenous origin and may account for the greater constancy of acetone and acetoacetic acid, assuming that ketone bodies, consisting predominantly of β-hydroxybutyric acid, are formed from ketogenic substances in the feed and/or ketogenic substances formed as the result of rumen digestion.

Although the increase in the blood and urinary acetone bodies during the stall feeding period was undoubtedly due primarily to silage feeding, it cannot be concluded with certainty that other factors were not involved. The fact that there was a continued increase throughout the entire stall feeding period suggests that other factors may have been involved. The data on the two-year-old heifers and the mature bull, however, indicate that the increased ketonemia and ketonuria in the cow group may have been due entirely to the ketogenic effect of silage.

The ketogenic effect of silage should not be misconstrued as indicating a relationship between grass silage feeding and clinical ketosis. In fact, it may well be that grass silage supplies nutritional substances needed for the prevention of clinical ketosis, as recent work (unpublished) indicates that pasture grass has a marked antiketogenic effect in cases of clinical ketosis.

SUMMARY

A marked increase in the concentration of total blood and urinary acetone bodies and their component fractions of acetone and acetoacetic acid, and β-hydroxybutyric acid was observed during the late winter and early spring months with animals receiving molasses-treated grass silage. The lowest concentrations of these substances were observed in these animals when they were on early spring and summer pastures and were receiving grain, hay, and only a relatively small quantity of silage.

It is believed that the relatively high values obtained for the group as a whole during the winter feeding period were due primarily to grass silage feeding. Although acetone and acetoacetic acid, and β-hydroxybutyric acid increased, the latter fraction was responsible for most of the rise. Since the feeding of fresh silage did not produce this effect, it appears that the increase in the acetone bodies on silage feeding was associated with the aging of the silage. In the light of the work of Bender *et al.* (2) and MacKay *et al.* (12) it is suggested that the increased production of the acetone bodies due to the feeding of aged molasses-treated grass silage is due primarily to the volatile acids in the silage.

Frequent observations on the blood and urinary acetone bodies of six cows showed a sharp decline in these substances immediately after the animals were placed on spring pasture. The blood and urinary acetone bodies had decreased 52.8 per cent and 51.5 per cent respectively after 16 days of daily pasture feeding. The maximum decreases in acetone and actoacetic acid were observed following the first day of pasture feeding. Blood and urinary β-hydroxybutyric acid, however, continued to decline throughout the 16-day period of observation. The sharp decline in acetone bodies after one day of pasture feeding was undoubtedly due primarily to the decreased silage feeding. The pasture grass feeding is believed to be partially responsible for the further continuous decline in the acetone bodies.

The blood acetone body levels of four heifers which were pasture fed during the summer months and received only mixed grass hay of a fair quality during the winter months were much lower while on hay feeding than on pasture feeding.

The blood acetone bodies increased following feeding, reaching a peak three hours after feeding, and then gradually decreased, returning to the pre-feeding level within nine hours. This effect was much more evident when aged grass silage was included in the ration.

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STUDIES ON KETOSIS IN DAIRY CATTLE. II. BLOOD AND URINARY ACETONE BODIES OF DAIRY CATTLE IN RELATION TO PARTURITION, LACTATION, GESTATION, AND BREED[1]

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A large percentage of the clinical cases of ketosis in dairy cattle occur during the first few weeks following parturition. This condition is associated with loss of appetite, a decrease in body weight, and a marked decline in milk production. Sjollema (10) early observed that ketosis frequently occurs during the first week to 10 days following parturition. Boddie (2) reported that ketosis usually occurs during the first six weeks following parturition and is frequently observed about two weeks after parturition. Duncan, Huffman, and Tobin (4) observed that the symptoms of ketosis were most marked two to six weeks following parturition in a herd of purebred Jersey cattle with a high incidence of ketosis.

Because of the large number of cases of ketosis observed shortly after parturition, emphasis was placed upon this particular period in the study of the normal level of the acetone bodies. Studies were also made of the effect of lactation, gestation, complete cessation of milking, and of breed upon the concentration of these substances in the blood and urine of dairy cows.

EXPERIMENTAL

During the course of this work the same precautions were observed in obtaining and analyzing the samples as were followed in the previous work (5). The method of Barnes and Wick (1) was used in the analyses of the acetone bodies.

Blood and urinary acetone bodies in relation to parturition and stage of lactation. In connection with the studies on the relationship of stall and pasture feeding to the level of blood and urinary acetone bodies (5), observations were also made of the levels of blood and urinary acetone bodies prior to and following parturition.

The data on 11 cows over a one-year period were plotted at 30-day intervals according to stage of lactation as shown in figure 1. There was an upward trend in the total urinary acetone bodies for a period of approximately

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[2] The experimental data in this paper are taken from a thesis presented by C. B. Knodt in partial fulfillment of the requirements for the degree of Master of Science in Dairy Industry, University of Connecticut, Storrs Agricultural Experiment Station.

90 days after parturition, followed by a gradual decline over the rest of the period. The total blood acetone bodies presented somewhat the same picture, although the trend was not as marked. A critical study of the data indicated that most of the rise during the first few months following parturition was due to the effect of grass silage feeding which results in a marked increase in blood and urinary acetone bodies (3).

Daily observations were made upon five of these cows for a period of three days prior to and including the day of parturition and for four days

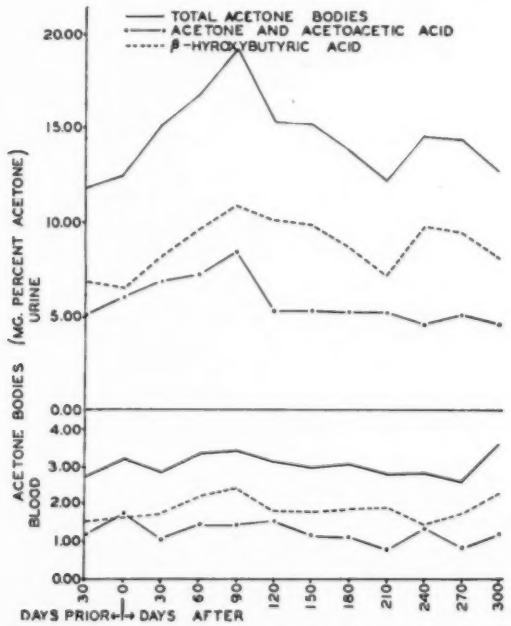

FIG. 1. Blood and urinary acetone bodies as related to stage of lactation. (Monthly analysis of 11 mature cows.)

following parturition. These data indicate a rather marked decrease in urinary acetone bodies during the four-day period following parturition. There also appeared to be a decline in blood acetone bodies, especially during the first two days following parturition. An analysis of variance of these data showed a highly significant ($F = 22.05$) decrease in total acetone bodies, and a significant ($F = 14.34$) decrease in urinary β-hydroxybutyric acid, but the average decrease in urinary acetone and acetoacetic acid was not significant ($F = 5.96$). A similar analysis was made on the blood acetone bodies and it was found that the apparent average decline during the four-day period following parturition was not statistically significant, the

F values being as follows: total acetone bodies ($F = 2.48$), acetone and acetoacetic acid ($F = 0.64$), and β-hydroxybutyric acid ($F = 1.18$).

Intensive studies upon the levels of blood and urinary acetone bodies of several cows prior to and following parturition showed large day-to-day variations. The results of such observations upon two cows are presented in figure 2. The day-to-day changes in blood and urinary acetone bodies are very large and while factors associated with parturition appear to affect the acetone bodies in certain cases any trend may be obviated by these variations.

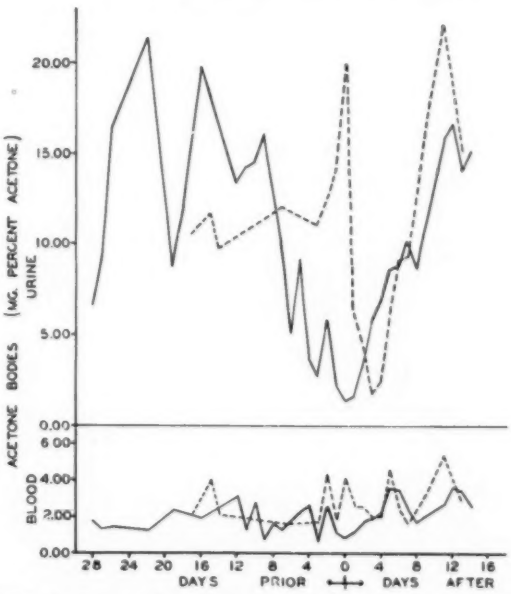

FIG. 2. Total blood and urinary acetone bodies of two mature cows prior to and following parturition.

The variations observed in the concentration of acetone bodies throughout the day at intervals of from two to four hours also showed large variations (5) although the magnitude of these changes was not quite as great as that observed prior to and following parturition. Although an attempt was made to continue the feeding of grass silage at the same level through the period of parturition, there were some variations in the consumption of silage during this period. While there may be a decrease over a relatively short period of time following parturition in some cases, it is deemed more significant that there is not a marked increase in blood and urinary acetone bodies during the period following parturition when the incidence of ketosis is greatest.

Blood and urinary acetone bodies during gestation. Blood and urinary analyses upon 10 cows at various stages of pregnancy are presented in figure 3. These data are quite irregular and indicate that factors other than pregnancy were affecting the concentration of blood and urinary acetone bodies. Undoubtedly some of the irregularity as well as the particular lack of an apparent trend is due primarily to normal day-to-day variations and to changes in feeding conditions.

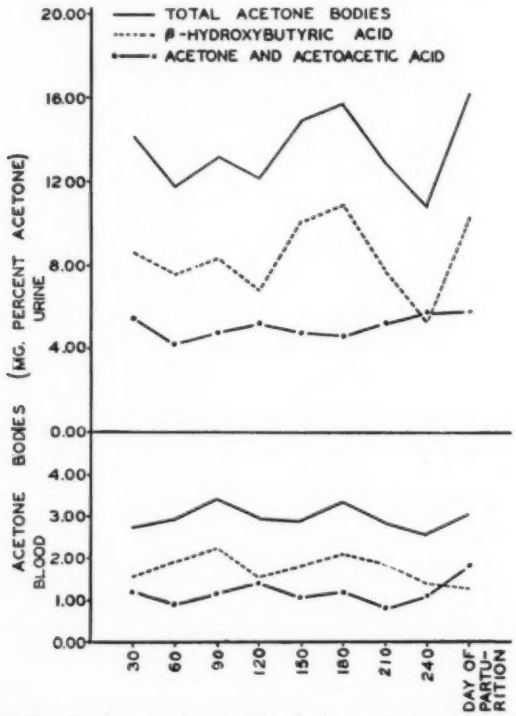

FIG. 3. Blood and urinary acetone bodies during gestation. (Monthly analysis of 10 mature cows.)

Blood and urinary acetone bodies prior to and following the complete cessation of milking. The utilization of β-hydroxybutyric acid by the lactating mammary gland was demonstrated by Shaw and Knodt (8). On the basis of our calculations a cow producing 20 kilos of milk per day must mobilize approximately 237.0 grams of β-hydroxybutyric acid per day for milk secretion. Because of this relatively large mobilization of acetone bodies for milk secretion purposes, a study was made to observe the effect of sudden cessation of milking upon the concentration of the acetone bodies in the blood and urine.

Five cows were studied for several hours prior to and following the complete cessation of milking. Normally these cows were milked at eight-hour intervals. It will be observed in figure 4 that there was no significant change in blood and urinary β-hydroxybutyric acid attributable to other than normal daily and hourly variations. The failure to observe an increase in the blood and urinary acetone bodies following the complete cessation of milking may be due to two factors: 1) that the gland continues to use β-hydroxybutyric acid for some time as in the case of glucose and amino acids (9), and 2) that there is an increased utilization of β-hydroxybutyric acid by the other tissues of the body.

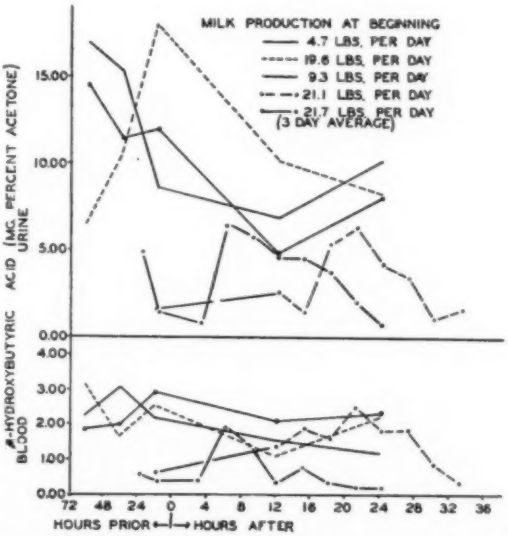

FIG. 4. Blood and urinary β-hydroxybutyric acid prior to and following the last milking.

Blood and urinary acetone bodies as related to breed. In the study of clinical ketosis at this station most of the cases have occurred in Jersey and Guernsey herds. The fact that more fat must be mobilized per unit of volume of milk produced by the cows of the higher testing breeds than those of the lower testing breeds suggests that the level of acetone bodies may be affected by breed. Since the production of acetone bodies by the liver may be expected to be proportional to the amount of fat catabolized, the cows were divided into two groups for purposes of comparison, in which one group was composed of four Guernseys and one Jersey and the other group was composed of four Holsteins and two Ayrshires. The data in figure 5 do not indicate any significant difference between the two groups

during the spring and summer months. During the winter months the acetone bodies of the higher testing breeds were higher than those of the lower testing breeds. It appears that the difference may have been due to a somewhat higher level of grass silage feeding per unit of body weight to the higher testing group. The production of acetone bodies due to the feeding

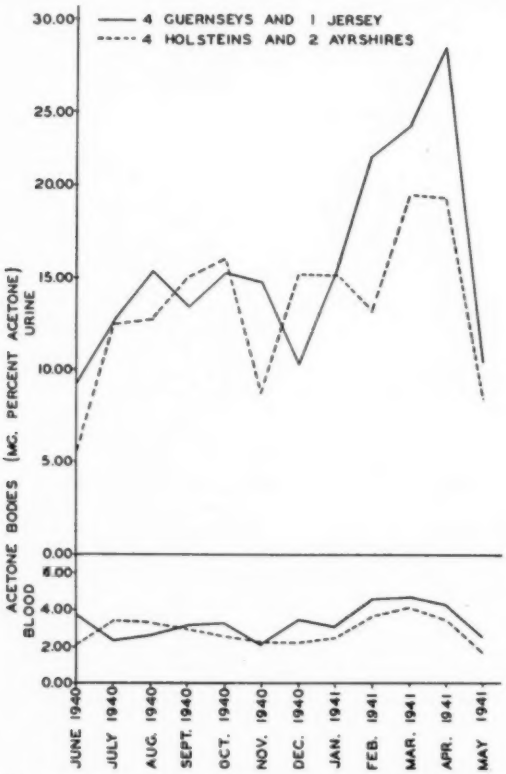

FIG. 5. Blood and urinary acetone bodies as related to breed and stall and pasture feeding.

of grass silage probably accounted for such a large proportion of the total acetone body production that any differences due to breed were obviated.

Comparison of blood, milk, and urinary acetone bodies. Sampson and Boley (7) have reported the analyses of milk from six cows following recovery from ketosis in which they were unable to detect the presence of acetone bodies by the method of Van Slyke (6). Duncan, Huffman, and Tobin (4) presented data on the concentration of acetone bodies in the milk of cows during recovery from ketosis. They were unable to detect acetone

bodies in the milk of several cows which appeared to have recovered from ketosis. Since we have shown that β-hydroxybutyric acid is used in substantial quantities by the mammary gland (8), the question arose as to whether all of the β-hydroxybutyric acid taken up by the gland is completely metabolized in the gland as in the case of blood glucose or whether some passes into the milk.

Analyses of 16 samples of blood, milk, and urine taken simultaneously are presented in table 1. These samples were analyzed for total acetone

TABLE 1

Blood, milk and urinary acetone bodies of mature dairy cows (16 simultaneously drawn samples)*

	Total acetone bodies	Acetone and acetoacetic acid	β-hydroxybutyric acid
Blood	3.14 ± 1.23†	1.17 ± 0.63	1.97 ± 1.25
Milk	1.80 ± 0.88	1.00 ± 0.63	0.80 ± 0.63
Urine	12.09 ± 6.09	4.24 ± 2.95	7.85 ± 3.87

* Expressed as mg. per cent acetone.
† Standard deviation.

bodies and the component fractions of acetone and acetoacetic acid and of β-hydroxybutyric acid. Both β-hydroxybutyric acid and acetone and acetoacetic acid were found in all the milk samples, although three samples contained only traces of the former. Although the level of β-hydroxybutyric acid was higher in the blood than that of acetone and acetoacetic acid, there was a lower concentration of this substance in the milk. This might be expected on the basis of previous work which indicated that β-hydroxybutyric acid is taken up by the active gland while the fraction composed of acetone and acetoacetic acid is not used. The concentration of acetone bodies in these 16 samples of milk was as follows: 1.80 ± 0.88 mg. per cent total acetone bodies; 1.00 ± 0.63 mg. per cent acetone and acetoacetic acid; and 0.80 ± 0.63 mg. per cent β-hydroxybutyric acid. Although the concentration of acetone bodies in milk was usually lower than that in blood, there were four cases where these were higher. There did not appear to be any correlation between the milk and blood acetone bodies within the normal range of these values.

A comparison of blood and urinary acetone bodies of mature dairy cows. In the course of the various studies of the normal level of blood and urinary acetone bodies, extending over a period of 15 months, a total of 427 samples of blood and urine were collected simultaneously.

The mean concentrations of the blood and urinary acetone bodies and the extreme ranges observed are presented in table 2. The average concentrations of total blood and urinary acetone bodies were 2.66 ± 1.23 and 11.81 ± 6.56 mg. per cent acetone respectively. β-hydroxybutyric acid made up 59.39 per cent of the total blood acetone bodies and 61.73 per cent of the total

TABLE 2

Blood and urinary acetone bodies of mature dairy cows (427 simultaneously drawn samples from 16 cows)*

	Mean	Standard deviation	Min.	Max.
Total blood acetone bodies	2.66	1.23	0.31	6.95
Blood acetone and acetoacetic acid	1.08	0.66	0.12	5.62
Blood β-hydroxybutyric acid	1.58	1.00	0.06	5.52
Total urinary acetone bodies	11.81	6.56	0.61	34.26
Urinary acetone and acetoacetic acid	4.52	2.85	0.04	19.78
Urinary β-hydroxybutyric acid	7.29	4.78	0.04	23.82

* Expressed as mg. per cent acetone.

urinary acetone bodies. No evidence of clinical ketosis or of other abnormalities were observed in these animals during the experimental period.

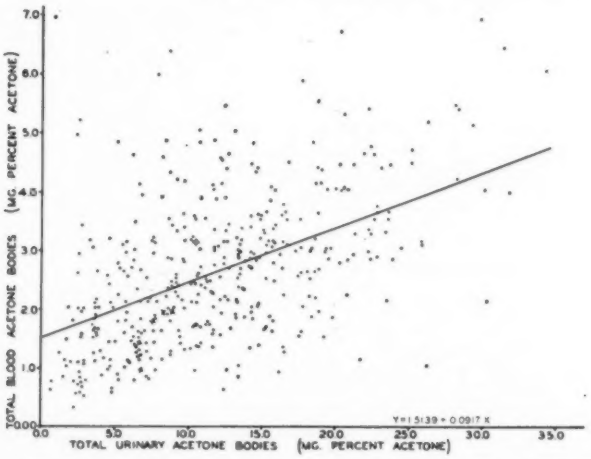

FIG. 6. Comparison of blood and urinary acetone bodies. (× = two separate values falling on the same point.)

The data presented in figures 6, 7, and 8 show a highly significant coefficient of correlation between the following blood and urinary substances: total acetone bodies ($r = 0.5115$), acetone and acetoacetic acid ($r = 0.3315$), and β-hydroxybutyric acid ($r = 0.3750$). It will be noted in figures 6, 7, and 8 that, although there was a significant correlation between the blood and urinary concentrations of these substances, there was a very large dispersion about the regression line.

There were highly significant coefficients of correlation between the total blood acetone bodies and the component fractions of acetone and acetoacetic acid ($r = 0.5920$), and β-hydroxybutyric acid ($r = 0.8377$). Simi-

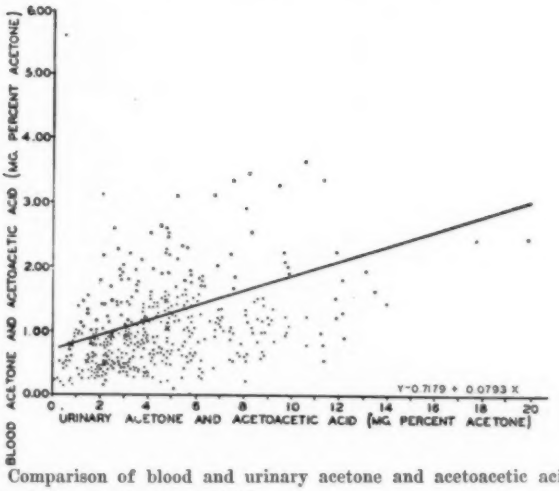

FIG. 7. Comparison of blood and urinary acetone and acetoacetic acid. (× = two separate values falling on the same point.)

larly there were significant coefficients of correlation between the total urinary acetone bodies and the component fractions of acetone and acetoacetic acid ($r = 0.7037$), and β-hydroxybutyric acid ($r = 0.9202$).

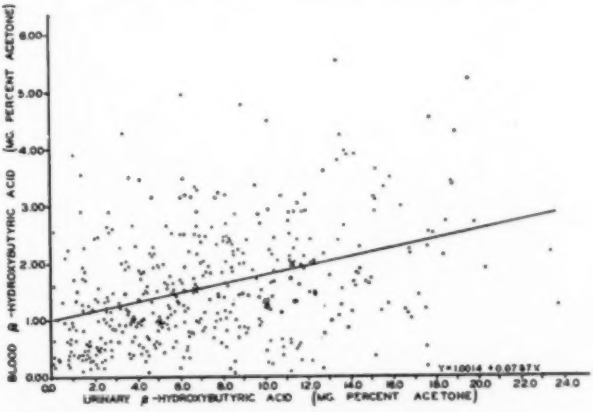

FIG. 8. Comparison of blood and urinary β-hydroxybutyric acid. (× = two separate values falling on the same point.)

CONCLUSIONS

The data presented in this paper indicate that factors such as stage of lactation, parturition, gestation, and sudden cessation of milking do not ordinarily exert much influence upon the concentrations of blood and

urinary acetone bodies and their component fractions of acetone and acetoacetic acid, and β-hydroxybutyric acid. If any of these factors had any effect upon the blood and urinary acetone bodies during these experiments, they were apparently obviated by large day to day and monthly variations due primarily to feeding conditions.

Acetone bodies were found in significant amounts in all of the milk samples analyzed but were usually lower in milk than in blood.

The mean concentration of total acetone bodies in 427 blood and urine samples obtained from 16 cows over a period of 15 months was 2.66 ± 1.23 and 11.81 ± 6.56 mg. per cent acetone respectively.

There was a highly significant correlation between the blood and urinary concentration of the following substances: total acetone bodies, acetone and acetoacetic acid, and β-hydroxybutyric acid.

The average composition of blood acetone bodies was 59.39 per cent β-hydroxybutyric acid and 40.61 per cent acetone and acetoacetic acid when calculated as acetone. The average composition of the urinary acetone bodies was 61.72 per cent β-hydroxybutyric acid and 38.28 per cent acetone and acetoacetic acid when calculated as acetone.

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STUDIES ON KETOSIS IN DAIRY CATTLE. III. BLOOD AND URINARY ACETONE BODIES AS RELATED TO AGE[1]

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In the studies of the normal variations of blood and urinary acetone bodies in dairy cattle, experiments were conducted to observe some of the variations in the concentrations of these substances in relation to age. A number of investigators (2, 6, 7) have suggested that rumen digestion may play an important part in the production of the acetone bodies. If the rumen is involved in the production of the acetone bodies, it might be expected that some differences would occur in the production of these substances with the changes that take place in the development of the rumen.

It also appeared that a relationship may exist between the concentration of the blood and urinary acetone bodies and the physiological processes related to production and reproduction.

EXPERIMENTAL

The following three age groups were studied: calves at birth, heifer calves from birth to 10 months of age, and heifers from 9 to 26 months of age. The same precautions were observed relative to the methods of obtaining samples and the analyses for acetone bodies as in previous work (4, 5). The method of Barnes and Wick (1) was used in the determination of the acetone bodies.

Blood acetone bodies from birth to 10 months of age. The concentration of total blood acetone bodies and the component fractions acetone and acetoacetic acid, and β-hydroxybutyric acid were determined upon eight calves at birth. Samples were also drawn from their dams at the same time for purposes of comparison. Table 1 reveals that the total blood acetone bodies

TABLE 1

Blood acetone bodies of eight calves and their dams following parturition*

	Calves	Dams	F (calves vs. dams)
Total blood acetone bodies	1.28 ± 0.65†	2.45 ± 0.63	21.92
Blood acetone and acetoacetic acid	0.71 ± 0.54	1.37 ± 1.04	6.10
β-hydroxybutyric acid	0.57 ± 0.82	1.06 ± 0.77	4.07

* Expressed as mg. per cent acetone.
† Standard deviation.

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[2] The experimental data in this paper are taken from a thesis presented by C. B. Knodt in partial fulfillment of the requirements for the degree of Master of Science in Dairy Industry, University of Connecticut, Storrs Agricultural Experiment Station.

of these calves at birth were considerably lower than those of their dams and of the normal values for mature cows previously reported (4, 5). An analysis of variance showed that this difference between calf and dam was highly significant. Because of the great variations in the composition of the total acetone bodies, there was not a highly significant difference between the calves and their dams in the concentrations of the two fractions.

There was no apparent correlation between the acetone bodies of the calves and their dams. This indicates a certain degree of independency of the calf in the production or utilization of the acetone bodies.

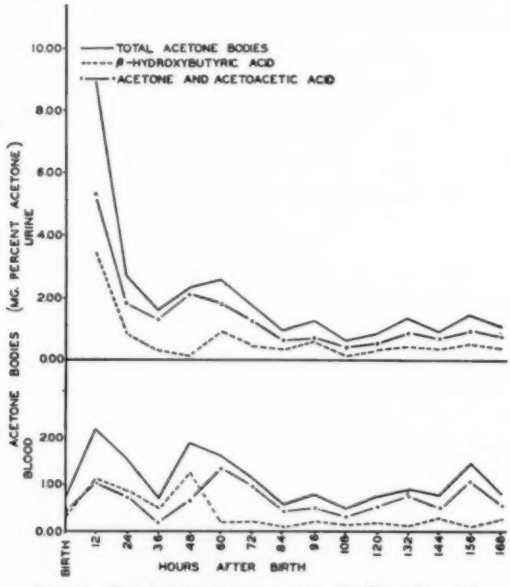

FIG. 1. Blood and urinary acetone bodies of a calf.

Blood and urinary acetone bodies and the component fractions, acetone and acetoacetic acid, and β-hydroxybutyric acid of a Jersey heifer calf were determined every 12 hours from birth to one week of age. The calf was allowed to remain with its dam for three days following birth, after which it was weaned and fed three pounds of whole milk twice each day. Blood and urine samples were taken at 6:30 A.M. and 6:30 P.M. and milk was fed at 8:00 A.M. and 4:00 P.M.

As indicated in figure 1, the blood and urinary acetone bodies were extremely low during the first seven days following birth. During this period most of the blood and urinary acetone bodies consisted of the acetone and acetoacetic acid fraction. During the first three days following birth

the values for blood and urinary acetone bodies were higher than the following four days. Although the morning samples were taken one and one-half hours before feeding and the evening samples two and one-half hours after feeding, there was no essential difference in the level of blood and urinary acetone bodies. This is of considerable interest since previous work (4) has shown a marked rise in blood acetone in mature cows following feeding.

Blood acetone bodies were determined at short intervals on four calves from birth to 10 months of age, and urinary acetone bodies were determined

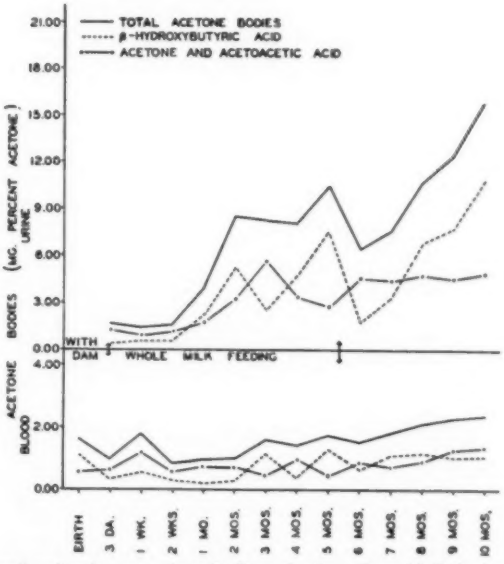

FIG. 2. Blood and urinary acetone bodies of calves from birth to ten months of age. (Average of 4 calves.)

on these calves from the third day after birth to 10 months of age. The results are shown graphically in figure 2. The blood acetone bodies were quite low for the first two months, after which there was a progressive increase extending over the entire period. The urinary acetone bodies were extremely low the first two weeks, being less than 2.0 mg. per cent. From the second week to the second month after birth the urinary acetone bodies increased about 400 per cent, and continued to increase with some fluctuations during the remainder of the period. It will be noted that the blood and urinary acetone bodies during the first few weeks were composed primarily of acetone and acetoacetic acid. After the third month the urinary β-hydroxybutyric acid fluctuated widely, whereas the acetone and acetoacetic acid remained more nearly constant. The increase in total urinary

acetone bodies from the seventh to the tenth month was due entirely to β-hydroxybutyric acid.

Blood and urinary acetone bodies from 9 to 26 months of age. Blood and urinary acetone bodies of five heifers were studied over a 12-month period. At the beginning of the experiment the heifers ranged from nine to 13 months of age. Three of the heifers were pasture fed during the summer and received hay and grain during the winter period. Two of the heifers were fed identically with the exception that they received no grain during the winter.

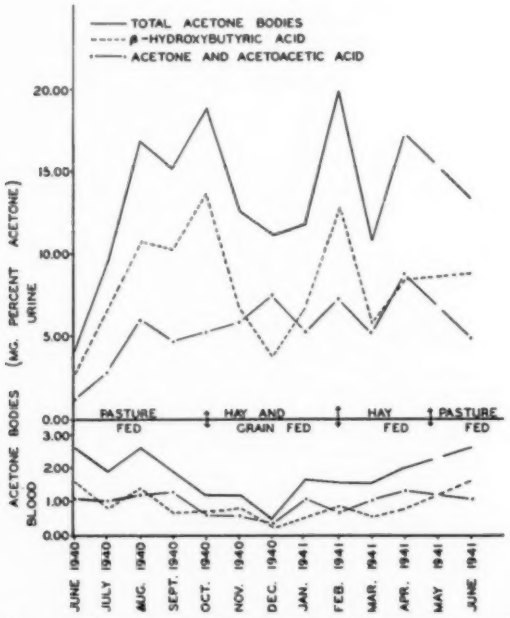

FIG. 3. Blood and urinary acetone bodies of 3 heifers 12 to 13 months of age as of June 1, 1940.

Since the feeding of grain appeared to have some effect upon the acetone bodies, the heifers were divided into two groups. As shown in figures 3 and 4, the blood acetone bodies of the three heifers receiving hay and grain during the winter period were markedly lower than those of the two heifers which received only hay during this period. As in the case of the older heifers on the same pasture (4), the blood acetone bodies were more concentrated while the heifers were on pasture than at any other period. This appeared to be in contradiction with previous experiments upon mature cows (4) in which it was observed that there was a decrease in the blood acetone bodies associated with pasture feeding.

The total urinary acetone bodies were extremely variable in these two groups of heifers and most of the variations in the total urinary acetone bodies were due to β-hydroxybutyric acid.

Comparison of blood and urinary acetone bodies of various age groups of dairy cattle. For purposes of comparison the data presented in table 2 show the relationship of age to the concentration of acetone bodies in blood and urine. Apparently the blood and urinary acetone bodies of the two youngest groups were considerably lower than for the older heifers and

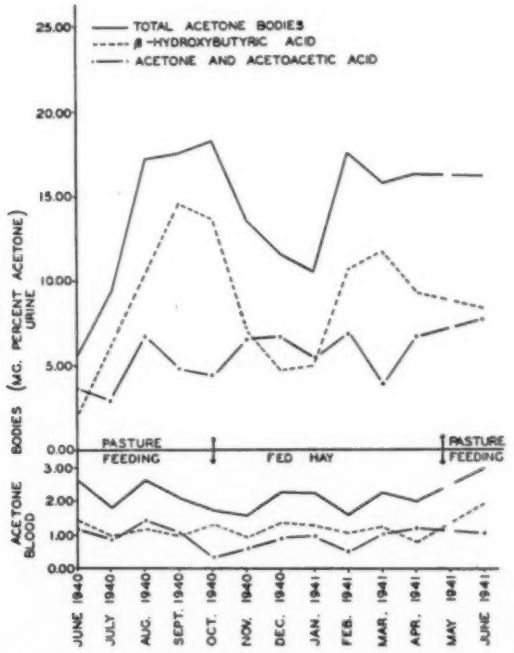

FIG. 4. Blood and urinary acetone bodies of 2 heifers 9 months of age as of June 1, 1940.

mature cows. The higher concentration of blood acetone bodies in the mature group as compared to the group 9 to 26 months of age may have been due to the effect of silage feeding to the former group. It would be expected that the urinary acetone bodies of the mature group would also be higher. This, however, was not true, as the urinary acetone bodies of the younger group were somewhat higher than those of the mature cows. The reason for this is not apparent.

The gradual increase in the acetone bodies of the calf during the first few months after birth appears to parallel roughly the development of the

TABLE 2

Comparison of blood and urinary acetone bodies of various age groups of dairy cattle*

Age group (months)	No. of analyses	Mean	S.D.	Min.	Max.
Total Blood Acetone Bodies					
Birth	8	1.28	0.75	0.31	2.43
Birth to 10	65	1.49	0.66	0.49	3.86
9–26	100	2.04	0.73	0.43	3.81
Mature	427	2.66	1.23	0.31	6.95
Blood Acetone and Acetoacetic Acid					
Birth	8	0.71	0.17	0.20	1.55
Birth to 10	65	0.81	0.11	0.17	1.46
9–26	100	0.98	0.45	0.10	2.29
Mature	427	1.08	0.66	0.12	5.62
Blood β-hydroxybutyric Acid					
Birth	8	0.57	0.84	0.01	2.23
Birth to 10	65	0.68	0.59	0.07	3.34
9–26	100	1.06	0.60	0.17	2.95
Mature	427	1.58	1.00	0.06	5.52
Total Urinary Acetone Bodies					
Birth	1	1.17	.....	.....	.....
Birth to 10	65	6.42	4.88	0.60	20.24
9–26	100	14.30	5.67	3.60	26.26
Mature	427	11.81	6.56	0.61	34.26
Urinary Acetone and Acetoacetic Acid					
Birth	1	0.93	.....	.....	.....
Birth to 10	65	2.94	1.97	0.20	5.40
9–26	100	5.31	2.48	0.34	11.44
Mature	427	4.52	2.85	0.04	19.78
Urinary β-hydroxybutyric Acid					
Birth	1	0.24	.....	.....	.....
Birth to 10	65	3.48	3.56	0.03	16.12
9–26	100	8.99	4.55	1.28	20.02
Mature	427	7.29	4.78	0.04	23.82

* Expressed as mg. per cent acetone.

rumen and indicates that the rumen may be responsible for the relatively large concentration of acetone bodies in the blood and urine of the bovine. However, during this period there is a gradual decrease in blood glucose (3) which may be associated with the increase in the level of blood and urinary acetone bodies. The high glucose level following birth may in turn be closely associated with the high liver glycogen content at this time (8), so that the apparent relationship between blood glucose and blood acetone bodies may be due to some extent to a more direct relationship between the liver glycogen and the blood acetone bodies.

CONCLUSIONS

Blood acetone bodies of calves from birth to two months of age were lower than at any other time. Urinary acetone bodies were extremely low for the first two weeks following birth after which they increased at a rapid rate.

Blood acetone bodies of the young calves were predominately acetone and acetoacetic acid, whereas β-hydroxybutyric acid usually made up the larger percentage in the older animals.

Heifers receiving pasture grass alone exhibited higher concentrations of blood acetone bodies than when receiving hay and grain. When hay alone was fed the blood acetone bodies were somewhat higher than when hay and grain were fed and somewhat lower than on pasture grass alone.

The concentration of acetone and acetoacetic acid in the urine was less variable than β-hydroxybutyric acid, the latter being largely responsible for the fluctuations in the total urinary acetone bodies.

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INFLUENCE OF pH, TYPE OF FAT, AND PANCREATIC EXTRACT UPON LIPOLYSIS IN HOMOGENIZED MILK*

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In a previous paper (2) results were presented on studies of lipolysis in homogenized milk and cream, which indicated that lipase activity in homogenized milk was not always affected to the same extent, if at all, by those factors which influence the enzymic action in unprocessed raw milk. The results presented herein constitute a continuation of these studies.

The influence of pH on lipase action in homogenized milk has been studied only to a limited extent although Dorner and Widmer (1) observed that acidifying homogenized raw milk with HCl retarded or even completely prevented rancidity development. However, in studies on non-homogenized milk, Mattick and Kay (4) found fat hydrolysis to be greatest at pH 8.2–8.7 and Roahen and Sommer (7) secured a somewhat similar optimum range of pH 8.4–8.6.

The non-specificity of milk lipase has been indicated by many workers in that certain specific fat acid esters have been utilized as the substrate for lipase action. Rogers, Berg and Davis (8) early used ethyl butyrate as a base for lipase action and more recently Mattick and Kay developed a method for measuring activity of the fat splitting enzyme in milk by its ability to hydrolyze tributyrin. Tarassuk and Palmer (9) indicate that milk lipase will hydrolyze the esters of lauric, myristic and palmitic acids. Later, Palmer and Hankinson (6) dispersed diglycol laurate, diglycol oleate, ethyl myristate and butyl stearate in raw skim milk by shaking, and secured hydrolysis in every case.

Hydrolysis of butterfat may be produced by enzymes from other sources than milk. Palmer (5) used a glycerine extract of pigs pancreas and later commercial steapsin to produce lipolysis. Tarassuk and Richardson (10) produced lipolysis in pasteurized cream with steapsin.

EXPERIMENTAL PROCEDURE

Conditions of processing were, in general, identical with those reported previously (2). Adjustments of pH were made with solutions of HCl and NaOH and determinations were by a Beckman pH meter and an E-type glass electrode.

Titrations for free fatty acids were conducted as before with the exception that a 10-gram sample and 0.05 N NaOH were used. One-half the

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values secured in this determination represent the acid degrees of the fat, *i.e.*, the milliliters of N NaOH per 100 grams fat.

In all cases where the acidity of the fat was determined, homogenization was at 500–700 pounds. In other trials, where acidity changes in the fat-skim milk emulsion were measured by titration, homogenization was at 2,500 pounds.

EXPERIMENTAL RESULTS

Influence of pH. Two series of trials were conducted with the view of ascertaining the influence of pH upon lipolysis. In the first series, purified butter oil was homogenized into rennet whey at 500–700 pounds pressure to secure a product containing approximately five per cent fat. Following homogenization, the mixture was divided into five lots which were treated as follows: Lot 1—control, pH approximately 6.5; Lot 2—adjusted to pH 4;

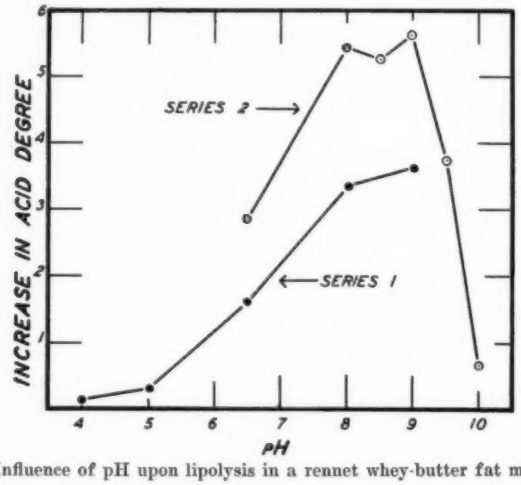

FIG. 1. Influence of pH upon lipolysis in a rennet whey-butter fat mixture.

Lot 3—adjusted to pH 5; Lot 4—adjusted to pH 8; Lot 5—adjusted to pH 9. After adjusting the samples to the desired pH, a portion of each was immediately pasteurized and fat secured for titration. The remainder was stored for 72 hours at 35–40° F. and the fat then analyzed to determine the extent of lipolysis.

In the second series, the same conditions prevailed with the exception that the pH was adjusted to 8, 8.5, 9.0, 9.5, and 10.

The results secured are shown by figure 1, in which the values are expressed on the basis of increases in acid degree over the 72-hour period. The maximum rate of lipolysis is within the range of pH 8 to pH 9. In Series 1, the results show no appreciable lipolysis at pH 4 and pH 5, and the results in Series 2 indicates great retardation of fat hydrolysis at pH 10.

The fact that lipolysis is practically prevented when the reaction is at pH 4 or pH 5 raises the question as to the permanency of this inhibition. To study this particular question, an experiment was conducted in which the pH of the fat-whey emulsion was adjusted to near the point of optimum lipase activity, *i.e.*, pH 8–8.5, after it had been at first subjected to pH 4. Then a determination was made to ascertain if any appreciable lipolytic activity persisted.

In the first series of trials in this connection, the casein was precipitated from raw skim milk with HCl by adjusting the reaction to approximately pH 4. The acid whey was then secured by filtration and was homogenized with butter oil to give a product containing about five per cent fat. This emulsion was then divided as follows into two lots: Lot 1—Acidity as secured from acid precipitation of casein, reaction approximately pH 4; Lot 2—Reaction adjusted to pH 8.5 with NaOH. Fat from a portion of each lot was analyzed at 0 and 72 hours. At the end of the 72-hour period, a portion of the lot held at pH 4 was then adjusted to pH 8.5 and held an additional 72 hours. Fat samples were secured immediately after making the change in reaction and also after 72 hours. The results of three trials are presented in table 1.

TABLE 1

The influence of low pH and subsequent readjustment to high pH upon the lipolytic activity of acid and rennet whey

Series 1—acid whey	Trial		
	1	2	3
	acid degree	*acid degree*	*acid degree*
(1) pH 4–4.4,* 0 hours	0.70	0.55	0.75
(2) pH 4–4.4, 72 hours	0.70	0.60	0.90
(3) pH 8.5, 0 hours	0.45	0.25	0.45
(4) pH 8.5, 72 hours	0.50	0.25	0.75
(5) Sample (2), adjusted to pH 8.5, 0 hours	0.50	0.75	0.55
(6) Sample (5) after 72 hours	0.40	0.30	1.30

Series 2—rennet whey	Trial 1†		Trial 2†	
	0 hours	72 hours	0 hours	72 hours
	acid degree	*acid degree*	*acid degree*	*acid degree*
Control—normal pH	1.49	2.56	1.35	3.88
Acid exposure—0 time	0.55	0.64	0.60	1.84
Acid exposure—1 hour	0.55	0.73	0.43	0.72
Acid exposure—18 hours	0.67	0.68	0.44	0.57

* Trials 1 and 2 were adjusted to pH 4, Trial 3 to pH 4.4.
† Trial 1 was adjusted to pH 3.7; Trial 2 to pH 4.13.

In the second series of trials, rennet whey was utilized instead of whey secured from acid precipitation of the casein. In this experiment, the whey was homogenized with butter oil to give a product containing approximately five per cent fat. Immediately after homogenization, a control sample was

secured. Fat from this sample was titrated for free fatty acids at once and again after 72 hours of storage. The remainder of the mixture was acidified to about pH 4 (pH 3.7 in one trial, pH 4.13 in another), and then divided into three lots. One lot was immediately adjusted to pH 8; another was held for one hour in the acidified medium and then adjusted to pH 8; the third was held about 18 hours in the acidified condition before it was adjusted to pH 8. In each case, one sample was secured for fat analysis immediately after adjusting to the alkaline range, and another after the mixture had been maintained for 72 hours in the alkaline condition. The results of this experiment are also presented in table 1.

These results, in general, indicate that acid treatment adversely and permanently affects the lipolytic activity of whey, at least under the conditions of this experiment. The acidified samples failed to respond to a marked degree with the alkali treatments, even though exposed to the acid treatment for only a few minutes. However, in those trials in which the reaction was maintained at a somewhat higher pH (Trial 3—Series 1, Trial 2—Series 2), the sample exhibited slight hydrolytic ability.

Type of fat. In another series of trials, a large number of different fats and oils were homogenized into raw and pasteurized skim milk to determine (a) the ability of milk lipase to produce fat hydrolysis in other than milk fat, and (b) the type of flavor produced. The results of two typical trials are presented in table 2.

TABLE 2

Lipolysis in various types of oils and fats when homogenized in raw skim milk

Type of sample	Increase in milliliters of 0.05 N NaOH for 18 gram sample during 96 hours			Flavor
	Trial 1	Trial 2	Avg.	
Control (skim milk)	0.10	−0.10	0.0	Normal
Cottonseed oil (2%)	1.60	1.10	1.35	.....
Cottonseed oil (4%)	1.80	1.30	1.55	Bitter, oily
Cottonseed oil (6%)	2.50	1.70	2.10	.....
Control (skim milk)	0.05	−0.10	−0.03	Normal
Castor oil (4%)	1.90	1.90	1.90	Bitter, oily
Coconut oil (4%)	3.00	1.90	2.45	Bitter, soapy, rancid-like
Corn oil (4%)	1.10	1.40	1.25	Bitter
Linseed oil (4%)	1.05	1.00	1.03	Bitter, oily
Olive oil (4%)	1.35	1.20	1.28	Bitter, oily
Control (skim milk)	0.0	−0.05	+0.03	Normal
*Margarine (4%)	1.3	1.00	1.15	Slight bitter
*Hydrogenated cooking fat (4%)	1.6	1.15	1.38	Slight bitter
*Lard (4%)	1.2	1.10	1.15	Slight bitter

* Melted at 100° F. before emulsifying in skim milk.

The results show appreciable fat scission to have occurred in each case when the fat was homogenized in raw skim milk whereas no such change was indicated in the pasteurized sample. Increases in the titer of 18-gram raw

samples containing fat varied from 1.0 to 3.0 ml. of NaOH for the different oils and fats studied.

All raw samples, with the exception of the control, developed a bitter flavor upon storage. The intensity of this flavor varied to some extent between different samples, with the oils producing a greater intensity of flavor than the solid fats. Because of the absence of the lower fatty acids in the substrates with the exception of coconut oil, the flavor which developed was not identical with the so-called rancid flavor of milk. However, the coconut oil sample gave a somewhat similar flavor.

Pancreatic extract. It is well known that milk fat may be hydrolyzed by lipolytic enzymes from various sources. The role homogenization may play in such action, however, has not been established. In this connection, trials were conducted in which unhomogenized and homogenized milk were treated with a commercial pancreatic extract. The pancreatic extract was one which has been recommended to be used for producing soft curd milk and as a preventive of oxidized flavor.

The pancreatic extract was placed in a water solution and then added at the rate of four grams to 100 pounds of milk. In one series of trials, the extract was added to raw milk before homogenization, in another, the extract was added to pasteurized milk following homogenization. In every trial, the milk was exposed to the action of the pancreatic extract for exactly 10 minutes after which it was either immediately homogenized and pasteurized or merely pasteurized, depending upon the experiment. Fat was secured from the samples and titrated for free fatty acids. The results are presented in table 3.

TABLE 3

Influence of pancreatic extract on lipolysis in unhomogenized and homogenized milk

Series No.	Treatment	Acid degree
1 (Raw milk)	Unhomogenized	0.60
	Pancreatic extract and unhomogenized*	1.06
	Homogenized†	1.65
	Pancreatic extract then homogenized*	11.06
2 (Raw milk)	Unhomogenized	0.83
	Homogenized†	1.58
	Homogenized*	2.95
	Homogenized—pancreatic extract*	10.54
3 (Pasteurized milk)	Unhomogenized	0.69
	Unhomogenized—pancreatic extract*	1.25
	Homogenized†	0.74
	Homogenized—pancreatic extract*	10.44

* Held 10 minutes before pasteurizing.
† Pasteurized immediately after homogenization.

As expected, the pancreatic extract produced lipolysis whenever it was used. However, the degree of lipolysis produced in the homogenized milk was phenomenal. Whether the extract was added prior to or subsequent

to homogenization made no distinguishable difference in the rate of its hydrolytic action. Neither did previous pasteurization of the milk affect the results, indicating that physical changes created by homogenization were the responsible factors. Acid degrees of the fat from homogenized milk to which pancreatic extract was added averaged about 10.7, whereas in the unhomogenized milk under the same conditions, the average acid degree was about 1.2. Naturally, homogenization of raw milk increased the free fatty acids, but this increase was relatively small in comparison to results secured when both homogenization and extract were used.

Rancidity was also produced in homogenized milk treated with pancreatic extract. Homogenization pressures of 500 and 2,500 pounds were both sufficient to produce rancid flavored milk when the milk was exposed to pancreatic extract action for 10 minutes. Furthermore, this flavor change occurred both in raw and pasteurized milk.

DISCUSSION

The fact that the optimum pH for lipase action in the homogenized whey-fat mixture was within the range of pH 8 to pH 9 indicates practically identical conditions in this respect between lipolysis as accelerated by homogenization and lipolysis in unprocessed or normal raw milk. This range agrees with the optimum values of pH 8.2–8.7 of Mattick and Kay (4) and pH 8.4–8.6 of Roahen and Sommer (7) for normal raw milk lipase. If there are several lipases in milk, as has been suggested (1, 3) and if the one which plays the principal role in homogenized milk is different from that which is the most prominent in the lipolytic activity of normal raw milk, then it does not appear logical to attempt to separate them on the basis of different optimum pH requirements.

The failure of acidified whey to exhibit appreciable lipolytic ability even though the pH was readjusted to the optimum pH range, indicates that the acidification permanently affects the fat hydrolyzing enzyme in milk. This is an important consideration if one attempts to concentrate or isolate milk lipase. On the basis of the results in this study, it would appear that any such attempt would require the reaction to be maintained in or near the alkaline range if appreciable enzymic activity is to be retained.

The studies with the various fats and oils indicate milk lipase possesses ability to hydrolyze a large number of different fatty substrates. The fats used varied widely in chemical composition and physical characteristics. However, all possess a certain proportion of unsaturated fatty acids which are likely responsible for at least an appreciable portion of the free fatty acids.

One point of interest in connection with these various fats is that in every case a bitter flavor was produced on lipolysis. Doubtless, a similar type of bitter flavor is produced in milk on the hydrolysis of butterfat but is often

overshadowed by the more typical rancid flavor produced by the lower fatty acids. Bitterness is frequently observed in so-called "rancid" milk, the bitterness apparently varying to a large extent between samples, perhaps because of the degree of lipolysis which has occurred. It is possible, therefore, that the rancid flavor of milk is due to a combination of two types of flavors: a putrid type caused by the freeing of butyric and perhaps other acids which will produce putridity and, secondly, a bitter type caused by freeing of certain other fatty acids capable of producing bitterness. The increase in titer of the fat is probably closely allied to the bitter producing fatty acids.

The interesting point relative to the studies with the pancreatic extract is the tremendous rapidity with which lipolysis is produced in the homogenized milk. When one considers that only a 10-minute exposure time is involved, then the extent of fat splitting becomes all the more remarkable. The question remains as to whether the action of the extract is favored by the smallness of the fat globules as produced by homogenization or by the change in the adsorbed layer around the newly created fat surfaces.

CONCLUSIONS

Optimum pH for lipase action in a homogenized rennet whey-fat emulsion was within the range of pH 8 to pH 9. Low pH values adversely and permanently affect lipase activity.

Milk lipase is a non-specific fat splitting enzyme capable of producing lipolysis upon a wide variety of fatty substrates under favorable conditions.

Homogenization creates a condition which greatly enhances lipolysis as produced by pancreatic extract.

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A COMPARISON OF RATS FED AN EVAPORATED MILK WITH THOSE FED A "MILK" IN WHICH THE NATURALLY OCCURRING FAT HAS BEEN REPLACED BY COCONUT OIL

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Milk substitutes are used in human nutrition and it is important to know their effect upon the body. One form of substitute for milk results from the addition of some other fat to skimmed milk. Several reports have recently appeared in the literature comparing the nutritive value of butterfat with that of various vegetable oils. Schantz, Elvehjem and Hart (15) showed an increased rate of growth in rats receiving butterfat as compared to those receiving corn, coconut, cottonseed or soybean oil homogenized in skimmed milk. Gullickson and Fountaine (8) reported results on calves which indicated superior nutritive properties for butterfat as compared to certain vegetable oils. Harris and Mosher (9) have reported a greater growth of coconut oil-fed adolescent rats than of those fed a similar diet containing butterfat, while Harris and Rosenfeld (10) reported a better growth of weanling rats fed butterfat than in those receiving coconut oil.

The present study is a comparison of the growth, bone ash, liver and tissue fat of rats fed an evaporated milk with animals receiving a "milk" in which the butterfat had been replaced by coconut oil. Both products were procured on the open market and, according to the claims of the manufacturer, contained similar amounts of vitamins A and D.

EXPERIMENTAL METHODS

Animals. All animals were of the Wistar strain and from the same colony. The sexes and litters were divided equally into two groups. All animals were caged separately and weighed every three days.

Analytical procedures. Liver fat was determined on the fresh organ by the method of Best, Channon and Ridout (1). The total and volatile fatty acids of the storage fat were determined on the alcohol-ether (3:1) extract of pooled peri-renal and mesenteric fat. The total fatty acids of the storage fat were estimated by titration, using a modification of the procedure described by Stoddard and Drury (16). On another aliquot of the alcohol-ether extract, the steam distillable fatty acids were determined, using the stills and technique described by Friedemann (5), but with the following adaptations: An aliquot of the alcohol-ether extract was transferred to the steam distilling flask and made up to a volume of 20 cc. with ethyl

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alcohol. One-tenth cc. of saturated sodium hydroxide was added and the extract evaporated to dryness on the steam bath. Fifteen cc. of distilled water was then added to the saponified residue and after the soaps had gone into solution the reaction was made acid to Congo red by the addition of M/1 phosphoric acid. The procedure from then on was as described by Friedemann except that the distillate of the second distillation was collected in an equal volume of 95 per cent ethyl alcohol. The final distillate was brought to a boil and titrated with freshly diluted N/100 sodium hydroxide, using phenolphthalein as an indicator. Generally, the distillate was collected in 100-cc. portions. Blanks were run on reagents and on unsaponified aliquots of the alcohol-ether extract. From these data the percentage of fatty acid acidity due to volatile fatty acids was calculated.

The percentage of bone ash was determined by ashing to constant weight at 500° C. after defatting the bones by continuous extraction with hot alcohol for 24 hours, followed by a similar extraction with ethyl ether.

Pooled samples of 3 cans of evaporated and "filled" milk were selected at random and analyzed by standard procedures (11) for fat, nitrogen, total solids and ash. On analysis the evaporated milk was found to contain 26.4 per cent solids, 7.74 per cent fat, 1.06 per cent nitrogen, and 1.70 per cent ash. Analysis of the "filled milk" gave the following results: total solids, 25.4 per cent, fat, 6 per cent., nitrogen, 1.32 per cent, and ash, 1.59 per cent. The evaporated milk was said to contain 325 U.S.P. units of vitamin D per can, and approximately 2000–3000 units of vitamin A, while the filled milk was said to contain 400 units of D and 2000 units of vitamin A.

EXPERIMENT AND RESULTS

The experiment was started when the rats were 25 to 28 days of age. Forty-three animals were fed evaporated milk and 42 animals received the product containing coconut oil. Approximately two-thirds the rats of each group were killed after 49 days on the experiment, and the remainder at 97 days. The two products were each fed to the respective groups of rats *ad libitum*. In addition, 0.25 mg. of iron, 0.05 mg. of copper and of manganese were fed separately as a supplement daily in a small amount of each food. The major portion of food was withheld until the supplement had been ingested.

The data obtained on these animals are summarized in table 1 and their growth curves are shown in figure 1. The growth curves for the animals carried for 97 days on the experiment are also included in the curves of the animals sacrified after 49 days. Growth on both evaporated and "filled" milk continued throughout the experiment. Growth was somewhat greater on the evaporated milk diet for both the 49- and 97-day animals. The difference in growth on the two diets is not statistically significant after 49 days, but is significant after 97 days (7). The percentage of bone ash is almost iden-

tical on the two diets, and the amount of liver fat is also similar. There is a definite difference in the amount of volatile fatty acids of the depot fat. The average value for the group given evaporated milk was 5.0 per cent of the total fatty acid acidity after 49 days on the diet, while the "filled milk" group gave a volatile fatty acid acidity equal to 16.3 per cent of the total storage fatty acid acidity. Hemoglobin determinations made on 10 rats from each group after 49 days on the experiment yielded similar results for each group.

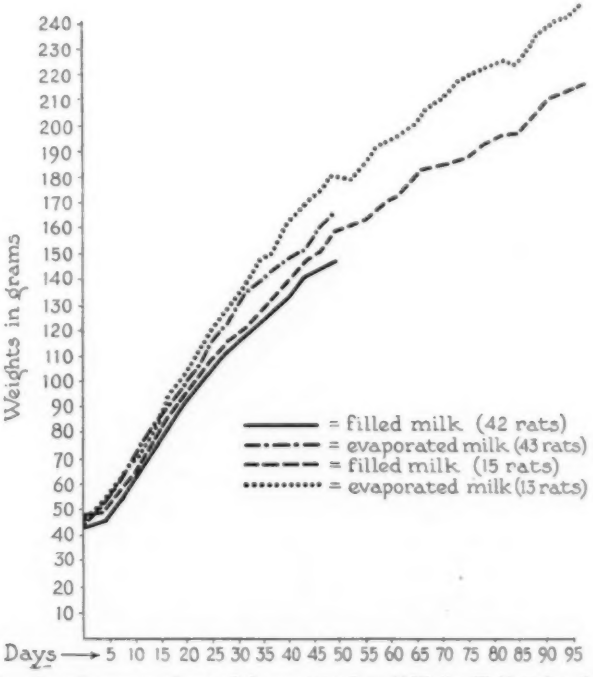

FIG. 1. Growth curves of rats fed evaporated or "filled milk" and a daily supplement of iron, copper and manganese.

During the last 48 days of the 97-day experiment, there was a greater incidence of loose stools passed by the "filled milk" rats than by the corresponding group fed evaporated milk.

Comment. Differences in growth observed in the present experiment cannot be ascribed to any particular aspect of the diet in as much as the diets differed in a number of respects. However, the most striking difference in the diet of the two groups of animals was in the character of the fat. The results of Schantz, Boutwell, Elvehjem and Hart (14) indicate that the

TABLE 1

Growth, percentage bone ash and liver fat of rats fed evaporated milk or a similar product containing coconut oil ("filled milk")

Milk	No. of animals on exp.	Days on diet	Average weights in gms.		Growth	Critical ratio* of growth differences
			Initial	Final		
Evaporated	43	49	43	160	116.9 ± 4.6†	1.64
Filled	42	49	43	149	106.7 ± 4.2	
Evaporated	13	97	46	247	201.0 ± 10.4	2.28
Filled	15	97	45	215	169.5 ± 9.0	

			% Bone ash	% Liver fat	Critical ratio	
					Bone ash	Liver fat
Evaporated	27	49	64.2	6.75	0.04	0.50
Filled	27	49	64.3	6.52		
Evaporated	13	97	67.4	.....	1.87	.....
Filled	15	97	66.6	.....		

* $C.R. = \frac{M_1 - M_2}{\sqrt{\sigma M_1^2 + \sigma M_2^2}}$. Where M_1 and M_2 are the mean values. σ = standard deviation of the mean.

† Standard deviation of mean in gms.

superior nutritive value of butterfat over corn oil resides in the saturated fraction of the fatty acids. The increased incidence of loose stools on the "filled milk" fed group carried for 97 days suggests that part of the difference in growth during the latter half of the experiment may have been due to a difference in gastro-intestinal tolerance for the two diets.

The effects of dietary fat upon the characteristics of the storage fat has been studied by many investigators. Gibbs and Agcaoili (6) showed that the physical and chemical constants of pig lard were altered by feeding the hogs on copra cake, which contains 7 to 15 per cent coconut oil. Apparently, fatty acid chains shorter than 10 carbon are not deposited in the tissues to a significant degree. Davis reported (3) some storage by chickens when tributyrin was injected, but none when it was ingested. Eckstein found no storage of butyric or caproic acids when they were fed to rats (14). Powell (13) recovered only traces of fed caprylic acid in the storage fat, while lauric acid was found up to 25 per cent of the fatty acids of the storage fat. When tricaprin was the sole fat in the diet of white rats, it was found that the storage fat contained 15 per cent capric acid (12). Coconut oil contains a high percentage of short-chained fatty acids (2) and

is particularly rich in lauric acid. The steam distillable fatty acids from the rats fed coconut oil was largely contained in the first 100 cc. of distillate. These fatty acids were water-insoluble and were solid at 15 degrees centigrade, so it seems reasonable to assume that they were lauric and capric acid. The volatile fatty acids of the butter fat fed animals were relatively low, and their identity is not apparent.

SUMMARY

The growth of rats fed an evaporated milk was greater over a 97-day period than for a similar group of rats maintained on a "filled milk" in which the butterfat had been replaced by coconut oil. The percentage of bone ash and of liver fat is quite similar for the two groups of rats, both after 49 and 97 days on the diets. There were more volatile fatty acids deposited in the storage fat of the coconut oil group.

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BACTERIOLOGICAL STUDY OF CHOCOLATE MILK*

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INTRODUCTION

The widespread use of chocolate milk in recent years is so well known that it is not necessary to enlarge upon the subject here. Mueller (12) investigated the bacterial flora of a number of commercially-packed chocolate syrups and cocoa powders, and reported that some of them contained large numbers of bacteria. It is obvious that the addition of a non-sterile substance to milk will mean the contamination of the milk. This would be particularly true of the syrups because chocolate milk made from them is not always subsequently heated; whereas, when a cocoa powder is used, the mixture is pasteurized to bring the powder into suspension.

It was the purpose of this study to investigate the effect of adding cocoa powders and chocolate syrups to milk on the bacterial content and keeping quality of the milk.

EXPERIMENTAL

I. *Bacteriological examination of cocoa powders and chocolate syrups*

The first experiment was a preliminary determination of the numbers and types of bacteria present in three brands of cocoa powders and three of chocolate syrups, preparatory to their being used to make chocolate milks. The powders and syrups were all commercially-packed products. Samples A and I of the cocoa powders were American-processed powders, and sample D was Dutch-processed. Of the syrups, samples B and T were made from cocoa powders, and sample S was a syrup flavored with an extract of cocoa. All of the containers were kept sealed and otherwise handled aseptically in order that the samples could be used for the subsequent experiments.

Bacterial counts were made on standard nutrient agar, and tomato agar (Kulp (7)) was employed for detecting the presence of yeasts and molds. Appropriate dilutions, determined by trials, were made in sterile distilled water, and 1-ml. portions of these dilutions were plated. All plates were made in duplicate. One series of duplicate plates was incubated at 37° C., and a parallel series of duplicate plates at room temperature (about 22° C. in the room used). The latter series was intended to estimate the numbers of bacteria present that would develop if the milk made from the products was left standing at room temperature. Bacterial counts were made at the end of 48 hours' incubation. Tomato-agar plates were incubated and examined up to 5 days if no mold or yeast growth appeared before that time.

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Streak cultures, from the suspensions used for plating, were made on blood-agar to detect the presence of hemolytic cocci, and on Endo's agar to detect gram-negative bacteria of intestinal types. These plates were incubated at 37° C., and examined at 24, 48, and 72 hours. Results of the whole experiment are shown in table 1.

TABLE 1

Bacterial counts per gram of cocoa powders and chocolate syrups (plates incubated at 22° and 37° C.)

	Nutrient agar		Endo's agar		Blood agar		Tomato agar*	
	22°	37°	22°	37°	22°	37°	22°	37°
Powder A	10,200	700	–	–	–	–	+	+
" D	80	120	–	–	–	–	+	+
" I	32,000	10,000	–	–	–	–	–	–
Syrup B	49,000	90,000	–	–	–	–	+	+
" S	30	50	–	–	–	–	–	–
" T	220	310	–	–	–	–	–	–

* Tomato agar was used to detect the presence of molds. Since mold growth, when present, covered the whole plate, no attempt was made to secure counts.

The numbers of bacteria in both powders and syrups showed wide variation. This was in agreement with the results published by Mueller (12). It will be noted, however, that no bacteria were recovered on the Endo plates, and no hemolytic organisms appeared on the blood-agar plates. This indicates that neither gram-negative intestinal bacteria nor hemolytic cocci were present in the powders or the syrups. A number of colonies growing on the nutrient-agar plates were examined both microscopically and culturally. Most of them proved to be aerobic spore-forming bacteria of the *Bacillus subtilis* group. Since all of these bacteria would have the same significance in milk—they could impair its keeping quality if they were present in sufficient numbers, but would not endanger the health of consumers—it was not considered necessary to identify them as to species. No gram-negative bacteria were isolated from the nutrient-agar plates, but a few gram-positive cocci were recovered. These were studied sufficiently to identify them as micrococci (Bergey (1)); but since very few of them were recovered, and they were probably of no great significance in milk, they were not identified as to species.

II. *Bacterial counts of chocolate milks made with cocoa powders and with chocolate syrups*

Whether the types of bacteria found in cocoa powders and chocolate syrups are pathogenic or not, substantial numbers of them could affect the keeping quality of chocolate milks. This would be especially true if the chocolate milk were kept at room temperature or at ineffective refrigeration temperature, as might be the case in some homes, or in carelessly operated public eating places.

The powders and syrups examined in the first experiment were used to make chocolate milks. Methods for the making of chocolate milk are not standardized, and the result is considerable variation in cocoa content among the products prepared by different market-milk plants. The milks in which cocoa powders were used were prepared according to the formula employed by the Department of Dairy Industry of the Massachusetts State College.

The powders were added by weight, and the milk was measured on the basis of 1.032 specific gravity. Cane sugar was added to each sample to make the final by-weight composition: 1 per cent cocoa powder, 7 per cent cane sugar, and 92 per cent milk. The ingredients were mixed and heated to 144° F. for 30 minutes to bring the cocoa into suspension. The mix was then cooled rapidly in an ice-water bath.

Chocolate milks were made from syrups by mixing cold pasteurized milk and cold syrup in the proportions recommended by the manufacturers of the respective brands of syrup employed. The mixtures were not heated.

After the chocolate milks had been prepared, each lot was divided into two portions. Plates were made immediately from all portions, using the medium and technic for the bacteriological analysis of milk according to Standard Methods (16). Then one portion of each milk was stored at room temperature, about 72° F. in the room used, and the other portion of each milk was stored in an electric refrigerator at about 43° F., "economy" temperature in the refrigerator employed. These two storage temperatures were employed to represent both satisfactory and unsatisfactory storage conditions. Subsequently, plates were made from all portions after about 7 hours' storage, and again after 24 hours. Plates were made again after 48 hours from the portions in the refrigerator. Two sets of duplicate plates were made each time from each portion, and one set was incubated at 37° C. and the other at room temperature. The use of 37° temperature is the usual practice, and room temperature was employed to obtain counts especially of those organisms that would develop as a result of room-temperature storage.

Circumstances did not permit carrying out the whole series of counts at one time. Consequently, check counts, such as those reported in table 1, were made from the several powders and syrups whenever they were opened to make fresh batches of chocolate milk. Freshly pasteurized batches of milk were used for each of the several preparations of chocolate milk that were necessary. Plate counts of milk were made each time it was obtained.

Results of this experiment are shown in tables 2 and 3. The numbers of bacteria added to milk with the addition of cocoa powders were not very important, except for spores, because the chocolate milk was heated to 144° F. for 30 minutes to bring the powders into suspension. This amounts to pasteurization. Further, only 1 per cent by weight of the milk is cocoa powder;

which would mean that, even when the highest-count cocoa powder was used (see table 1), the bacterial count of the milk would be increased by only a few over 100 per ml. Probably the pasteurization would destroy or inactivate many of these organisms. When syrups were used, however, around 10 per cent by weight of syrup was added to milk, and the mix was not heated. Thus, the bacterial content of the chocolate milk was substantially increased when high-count syrup was used, as can be seen by noting the counts for chocolate milk made with syrup B in tables 2 and 3.

TABLE 2

Bacterial counts of chocolate milks stored at refrigerator temperature (43° F.)

Sample	Plates incubated at 37° C.				Plates incubated at 22° C.			
	Hours of storage				Hours of storage			
	0	7	24	7 days	0	7	24	7 days
Powder A								
Milk	1,150	1,130	1,180	8,100	1,200	1,100	1,400	17,000
Chocolate milk	1,110	1,140	1,180	1,430	1,000	1,100	1,510	3,000
Powder D								
Milk	760	1,220	1,380	7,500	1,100	1,090	1,120	16,400
Chocolate milk	610	800	840	1,300	980	970	970	8,000
Powder I								
Milk	1,600	1,710	1,700	3,300	1,700	3,570	3,200	16,500
Chocolate milk	1,700	1,690	1,640	2,110	1,320	3,400	3,600	5,900
Syrup B								
Milk	1,610	1,580	1,650	4,000	1,660	1,620	2,610	9,000
Chocolate milk	8,400	8,700	8,600	12,300	6,400	6,900	8,400	9,800
Syrup S								
Milk	1,000	1,000	1,600	3,100	1,200	1,000	1,600	2,000
Chocolate milk	800	1,400	2,400	4,000	1,400	1,500	1,550	2,600
Syrup T								
Milk	1,390	1,370	4,100	6,000	2,400	2,300	2,900	13,000
Chocolate milk	1,330	1,400	3,100	3,900	2,200	2,100	2,600	3,100

For all of the milks made from cocoa powders, the counts had not increased much at the 7-hour period, and all of the 24-hour counts were substantially lower than the counts for the corresponding milk samples at room-temperature storage; this was also true for the 7-day counts of samples stored in the refrigerator. This fact indicates that the cocoa powders contained some factor that inhibited bacterial growth in chocolate milks made from them. Counts from chocolate milk made with syrup T showed the same trend as did counts from the milks made with cocoa powder. Syrup B had a high initial count, so the milk made with it had a high initial count compared with the count from the corresponding milk sample, and the count from the chocolate milk remained relatively high throughout. It will be noted, however, that the percentage increases of counts from this chocolate milk were definitely lower than were those from the corresponding milk

sample, for both conditions of storage and incubation. These facts indicate that both syrups B and T contained the same growth-inhibiting factors that were present in the cocoa powders. The counts from the chocolate milk made with syrup S followed closely those fro n the corresponding milk samples. This syrup was flavored with an *extract* of cocoa powder, and had no

TABLE 3

Bacterial counts of chocolate milks stored at room temperature (72° F.)

Sample	Plates incubated at 37° C.			Plates incubated at 22° C.		
	Hours of storage			Hours of storage		
	0	7	24	0	7	24
Powder A						
Milk	1,900	4,000	4,500,000	2,400	2,800	20,000,000
Chocolate milk	2,000	3,100	1,700,000	2,400	2,200	8,000,000
Powder D						
Milk	1,300	1,800	6,000,000	1,210	1,750	16,500,000
Chocolate milk	1,800	1,850	2,300,000	860	1,790	3,000,000
Powder I						
Milk	1,220	1,250	4,100,000	1,890	7,500	30,000,000
Chocolate milk	1,300	2,400	2,600,000	1,330	4,600	17,000,000
Syrup B						
Milk	1,760	1,740	1,600,000	1,800	1,890	14,500,000
Chocolate milk	7,800	8,500	1,680,000	9,400	8,400	16,900,000
Syrup S						
Milk	1,290	1,500	2,100,000	1,210	2,800	20,000,000
Chocolate milk	1,300	1,400	1,800,000	1,800	2,200	16,000,000
Syrup T						
Milk	1,340	1,810	3,000,000	1,410	2,100	43,000,000
Chocolate milk	1,000	1,700	1,500,000	1,300	1,700	18,000,000

cocoa in it, so the growth-inhibiting factor was evidently lost in its manufacture.

III. *Growth of certain species of bacteria in chocolate milk*

This experiment was set up to study the growth of certain bacterial species in chocolate milks. The species selected for study were among those that might be found in milk under ordinary conditions, and no specifically pathogenic bacteria were included. The bacterial species were: *Escherichia coli, Aerobacter aerogenes, Staphylococcus aureus, Streptococcus lactis,* and *Bacillus cereus.*

The chocolate milks were made as in experiment II; and the same powders and syrups were used, except that syrup S was omitted because it had not inhibited bacterial growth in the experiment. After the milks had been made up, they were sterilized in the autoclave, and were then inoculated from 24-hour cultures of the respective bacterial species. Duplicate series were stored as before at room temperature and at refrigerator "economy"

temperature. Platings were made immediately after the milks had been inoculated, at about 7 hours, and at 24 hours. Platings were made also at 48 hours from the milks stored in the refrigerator. Because of the volume of work involved, and because room-temperature incubation is not standard according to Standard Methods of Milk Analysis, plates were incubated only at 37° C.

Results are shown in table 4. It will be noted that inoculations were

TABLE 4

Growth of pure cultures of bacteria in chocolate milk (plates incubated at 37° C.)

	Refrigerator storage				Room storage		
	Hours of storage				Hours of storage		
	0	7	24	48	0	7	24
E. coli							
Milk	980	880	1,190	1,220	1,110	28,100	200,000,000
" + sucrose	1,070	.....	1,430	1,520	1,170	18,600	216,000,000
A*	920	1,070	1,090	820	930	10,100	85,000,000
D	1,120	1,170	1,180	630	1,200	12,500	93,000,000
I	950	1,110	1,100	560	1,110	15,400	80,000,000
B	990	950	900	650	1,020	6,000	69,000,000
T	900	770	410	290	920	2,200	6,000,000
A. aerogenes							
Milk	1,270	1,300	1,350	1,240	1,360	18,300	297,000,000
" + sucrose	1,310	1,240	1,290	1,180	1,280	14,100	198,000,000
A	1,340	1,290	1,210	1,150	1,310	1,310	39,200,000
D	1,390	.....	1,120	1,170	1,270	1,890	63,100,000
I	1,200	1,150	1,270	1,180	1,330	1,980	91,000,000
B	1,350	.....	1,230	1,150	1,230	1,570	5,300,000
T	1,250	1,130	1,130	1,110	1,210	1,270	990,000
Staph. aureus							
Milk	6,420	6,370	4,010	2,630	6,390	37,700	35,000,000
" + sucrose	6,610	6,560	3,730	2,150	6,170	35,600	60,000,000
A	5,640	5,710	520	412	5,960	12,300	1,600,000
D	6,120	5,820	1,750	1,670	5,890	21,000	4,900,000
I	6,530	6,180	750	360	5,990	10,700	2,100,000
B	6,370	6,280	1,820	1,220	6,440	9,800	110,000
T	6,100	5,420	970	1,180	6,070	6,300	108,200
Strep. lactis							
Milk	2,200	2,250	2,320	2,710	2,300	84,200	170,000,000
" + sucrose	2,370	2,150	2,080	2,320	2,280	87,600	165,000,000
A	2,120	1,890	1,940	2,130	2,420	75,400	260,000,000
D	2,040	1,720	2,300	2,610	2,010	67,300	149,000,000
I	2,160	1,570	1,790	2,260	2,450	83,200	273,000,000
B	2,060	1,410	1,600	1,670	2,030	37,500	132,000,000
T	2,210	1,480	1,540	1,610	2,460	27,000	126,000,000
B. cereus							
Milk	1,580	1,810	1,950	2,000	1,780	14,800	42,000,000
" + sucrose	1,610	1,590	1,600	2,000	1,800	13,100	50,000,000
A	1,560	1,550	1,800	1,200	1,850	4,510	17,000,000
D	1,650	1,470	1,590	1,400	1,720	4,560	15,000,000
I	1,730	1,580	1,700	1,430	1,810	3,860	22,000,000
B	1,590	1,540	1,680	1,110	1,860	4,420	7,000,000
T	1,600	1,610	1,700	1,000	1,800	3,220	4,000,000

* Letters represent chocolate milks made with cocoa powders A, D, and I, and chocolate syrups B and T.

made in milk containing the amount of sugar (sucrose) used in making the cocoa-powder mixes. This was done to determine the effect of the sugar concentration on bacterial growth. The results of the experiment may be summarized as follows:

Refrigerator storage. No particular effect on the numbers of coliform bacteria was noticeable within 24 hours. At 48 hours the number of *Escherichia coli* were somewhat diminished in all of the chocolate milks, while counts of *Aerobacter aerogenes* were not affected. The counts of *Staphylococcus aureus* decreased, even in the milk, in 24 hours, and they decreased still further in 48 hours. The counts of the organism were lower in the chocolate milks than in the milks without syrup or powder. *Streptococcus lactis* apparently was not much inhibited in any of the milks, although the counts in the milks with syrups B and T were somewhat lower than the counts in the other milks at both 24 and 48 hours. All of the counts of *Bacillus cereus* in chocolate milk, at 48 hours' storage, were lower than those of the milk controls. This result was unexpected in view of the fact that most of the bacteria found in the syrups and powders, in experiment I, were members of the *Bacillus subtilis* group. The explanation is not immediately apparent.

Room storage. Except for *Streptococcus lactis,* the counts of the several bacterial species employed were definitely lower from the chocolate milks than they were from either the control milk or the milk with sugar. In most instances, however, the counts were somewhat increased at 7 hours, and definitely increased at 24 hours. From the milk made with syrup T, counts of all the bacterial species, except *Streptococcus lactis,* were substantially lower than from the other chocolate milks. The same was true of counts of *Aerobacter aerogenes, Staphylococcus aureus,* and *Bacillus cereus* from milk made with syrup B.

In general, it may be stated that, with the exception of some of the counts of *Streptococcus lactis,* the results of the experiment were consistent with those shown in tables 2 and 3, in that they indicated the presence in the chocolate milks of some agent that inhibited bacterial growth. The counts from the milk with added sucrose were about the same as in the milk without it, which indicated that the inhibition of bacterial growth was not attributable to the increased sugar content of the chocolate milks.

IV. *Growth of bacteria in the presence of certain constituents of cocoa and chocolate*

The second and third experiments provided data which suggested that all three of the cocoa powders and two of the chocolate syrups possessed some power to inhibit bacterial growth in chocolate milks made with them. The fourth experiment was set up to attempt to discover the factor or factors responsible for the observed inhibition.

Analyses of cocoa products (Kuzmeski and Mueller (8); Whymper (18))

have shown that tannic substances, theobromine, and oxalic acid are present in sufficient concentrations to suggest that they might be able, singly or in combinations, to affect the rate of bacterial growth in chocolate milk. The concentrations of these compounds vary in different cocoas; but, in general, their approximate percentages seem to be: Tannic substances, 10 per cent; theobromine, 2 per cent; and oxalic acid, 0.65 per cent. In the formula, given in experiment II, for making chocolate milk from cocoa, one per cent by weight of cocoa is added to milk. On that basis the chocolate milk would contain 0.1 per cent of tannic substance, 0.02 per cent of theobromine, and 0.0065 per cent of oxalic acid.

The nature of the tannic substances in cocoa powders, and consequently in chocolate syrups, is not known, but tannic acid is probably not present to any great extent as such. That was the only tannin derivative, however, that was available in pure chemical form for use in the experiment.

Freshly pasteurized milk from the college dairy was used, as before, for the culture medium. The desired quantities of tannic acid, theobromine, and oxalic acid were weighed and added to 100-ml. portions of the milk, and these portions were again pasteurized. The tannic acid, theobromine, and oxalic acid were added, separately and in combination, in amounts that would give approximately the same concentrations as those indicated in the analyses previously mentioned. Sugar (sucrose) was used also with the combined compounds to indicate its effect, if any, on bacterial growth in the presence of the compounds. The same bacterial species were employed as in experiment III, and they were inoculated into the milk and milk-mixes in the same way. Room-temperature storage was employed. Platings were made immediately after the inoculations, and at 7 hours and 24 hours of storage. Plates were incubated at 37° C., and were counted at 48 hours. Results are shown in table 5.

The results obtained indicate that tannic acid alone, and the combination of all three compounds, inhibited the growth of the coliform bacteria, of *Staphylococcus aureus,* and of *Bacillus cereus,* but not of *Streptococcus lactis.* These results agree with the effect observed in experiment III, in which cocoa powders and chocolate syrups were used. Neither oxalic acid nor theobromine alone had any demonstrable effect on bacterial growth. The combination of all three compounds did not appear to have any greater inhibiting property than did the tannic acid alone, except in the combination with added sugar. With the latter combination *Streptococcus lactis, Escherichia coli,* and *Bacillus cereus* all gave lower counts than with tannic acid alone. The general trend of the counts obtained in the experiment indicates that tannic acid was the principal factor in inhibiting bacterial growth. If the action of tannic acid can be accepted as being representative of the action of tannin compounds, these results argue in favor of the tannic substance present in cocoa being the significant factor in the inhibition of bacterial growth observed in chocolate milks.

TABLE 5

Growth of pure cultures of bacteria in the presence of tannic acid, theobromine and oxalic acid (plates incubated at 37° C.)

Milk, and milk with added substances	Hours of storage at room temperature		
	0	7	24
E. coli			
Milk	3,280	56,300	553,000,000
Sugar, tannic acid, oxalic acid, theobromine	2,940	10,700	76,000,000
Tannic acid, oxalic acid, theobromine	1,910	10,200	291,000,000
Tannic acid	3,000	6,700	261,000,000
Theobromine	3,280	108,000	641,000,000
Oxalic acid	2,960	90,000	582,000,000
A. aerogenes			
Milk	4,040	62,000	574,000,000
Sugar, tannic acid, oxalic acid, theobromine	4,320	27,000	74,000,000
Tannic acid, oxalic acid, theobromine	4,610	21,000	53,000,000
Tannic acid	4,930	26,000	85,000,000
Theobromine	4,870	57,000	392,000,000
Oxalic acid	3,890	64,000	479,000,000
Staph. aureus			
Milk	1,890	17,680	40,300,000
Sugar, tannic acid, oxalic acid, theobromine	1,810	14,870	13,370,000
Tannic acid, oxalic acid, theobromine	1,790	13,960	12,110,000
Tannic acid	1,920	13,280	10,980,000
Theobromine	1,970	18,820	51,400,000
Oxalic acid	1,830	16,240	38,600,000
Strep. lactis			
Milk	1,710	.....	61,000,000
Sugar, tannic acid, oxalic acid, theobromine	1,550	8,900	25,000,000
Tannic acid, oxalic acid, theobromine	1,730	14,900	44,000,000
Tannic acid	1,790	16,900	64,000,000
Theobromine	1,670	13,300	68,000,000
Oxalic acid	1,840	18,800	76,000,000
B. cereus			
Milk	1,780	9,300	7,100,000
Sugar, tannic acid, oxalic acid, theobromine	1,520	8,900	2,600,000
Tannic acid, oxalic acid, theobromine	1,560	7,100	6,200,000
Tannic acid	1,580	11,100	5,300,000
Theobromine	1,650	10,300	7,000,000
Oxalic acid	1,620	17,600	6,400,000

DISCUSSION

The absence of potentially pathogenic bacteria from the cocoa powders and chocolate syrups, examined in this study, agrees with the results reported by Mueller (12). The absence of these types of bacteria is reassuring so far as the safety of chocolate milk is concerned. This is particularly true with respect to gram-negative bacteria of intestinal type, as it indicates that there is little danger of intestinal bacteria, and of typhoid and dysentery organisms particularly, being added to milk when chocolate milk is made. The fact that the growth of bacteria, excepting *Streptococcus lactis*, was inhibited in chocolate milks provides an additional margin of safety. An exception would be a chocolate milk made from an extract-flavored syrup such as syrup S.

Kliewe and Strawe (5) reported that cocoa products have definite germicidal power, and they added that this is the reason why "contamination of cocoa with intestinal bacteria has never been reported." The results of the present experiments do not argue in favor of the powders and syrups having sufficient germicidal power to render an infected milk safe by their addition to it, but there would seem to be little danger that milk would be dangerously contaminated from the addition of cocoa products, or that the bacterial count would be substantially increased in the chocolate milk unless a powder or syrup of unusually high bacterial content was used.

Tannic acid added to milk definitely inhibited bacterial growth in milk. This was true when tannic acid was used alone or in combination with theobromine and oxalic acid. The effect of tannic acid on bacteria has been studied by several investigators. Taylor (17) found that only small concentrations of tannic acid were required to inhibit growth of pyogenic cocci. Ko (6) stated that tannic acid was a more effective germicide for typhoid bacilli than were other fruit or vegetable acids. Olitsky and Cox (14) treated the nasal passages of mice with tannic acid and found them resistant to subsequent nasal infection with equine encephalomyelitis virus; and Sabin, Olitsky, and Cox (15) found that monkeys similarly treated were resistant to subsequent nasal infection with poliomyelitis virus.

As was stated before, the nature of the tannin complex in cocoa products is not known, and there may be some question as to how accurately the use of tannic acid may represent the action of the tannin complex. Perhaps a partial answer to this question may be found in the work of Doelger (2, 3, 4), who reported that bacteria in tan liquors are largely acid-producing organisms, with lactic, acetic, and butyric-acid producers in greatest abundance. *Streptococcus lactis* was the chief producer of lactic acid. Coliform bacteria have been found in weak tan liquors, but chromogenic bacteria have seldom been encountered. These results compare favorably with those of the present study in that the growth of *Streptococcus lactis* was not inhibited in either chocolate milks or in the presence of tannic acid, but the growth of coliform bacteria and *Staphylococcus aureus* was inhibited in both situations. The results of the present experiments suggest that the tannin complex of the cocoa products was the active factor in the inhibition of bacterial growth.

Lipman and Mueller (9) reported that when a ration containing milk powder and cocoa powder was fed to albino rats, the digestibility of the milk protein was reduced in comparison with its digestibility in rats that received no cocoa powder. In a similar study, Mueller and Ritchie (13) found that cocoa powder in the diet retarded the growth of albino rats. Mueller (11) reported that theobromine (0.27 per cent) in a diet was nontoxic for rats; 2 per cent crystalline tannic acid was toxic; and the toxicity of cocoa powders was related to their quantity of tannic substance. Toxicity was measured by growth of the rats. Mehlitz and Maass (10), and Zlatarov

and Poppov (19) reported that tannic acid, even in small quantities, had an inhibiting effect on enzymes. While the several studies mentioned are not concerned with bacteria, they do suggest a certain parallel with the results obtained in the present study.

It was interesting to note that the palatability of the chocolate milks employed was parallel to their bacteria counts. All samples of chocolate milk stored in the refrigator were palatable at 48 hours, as was to be expected. All of these samples were kept in the refrigator and tasted again at the end of 7 days. All of the samples made with the cocoa powders, and that made with syrup T, were still palatable. The respective milk samples, and the chocolate milks made with syrups B and S, were slightly rancid. Samples stored at room temperature were tasted at 24 hours, and all were palatable except those made with syrups B and S. These were definitely unpalatable at this time, as were the respective control milk samples. The spoilage of the milks made with the two syrups can be accounted for by the fact that syrup B had a high initial bacterial count, and syrup S was flavored with a cocoa extract. The chocolate milks made with the syrups were not pasteurized after they were mixed as were the milks made with the cocoa powders.

SUMMARY

A bacteriological study was made of chocolate milks containing, respectively, three brands of cocoa powder and three of chocolate syrup.

A preliminary experiment showed that two of the powders and one of the syrups had high bacterial counts, and the others had low counts.

Growth of the bacterial flora of the milk itself was inhibited when powder or syrup was added, except with one syrup (S) which was flavored with an extract of cocoa powder.

Growth of pure cultures of bacteria was inhibited in the chocolate milks, excepting growth of *Streptococcus lactis*. Other species of bacteria employed were *Escherichia coli*, *Aerobacter aerogenes*, *Staphylococcus aureus*, and *Bacillus cereus*. Syrup S was not used in this experiment.

Tannic acid added to milk inhibited the growth of the same bacterial species as those mentioned in the preceding paragraph, and the growth of *Streptococcus lactis* was not hindered.

Parallel groups of samples were stored at room temperature (72° F.) and at refrigerator temperature (43° F.) for the experiments on the milk flora and pure cultures in chocolate milk. For the experiment with tannic acid, oxalic acid, and theobromine, only room-temperature storage was used. The inhibition of bacterial growth at room temperature was more marked because of the greater growth-rate of bacteria at that temperature as compared with refrigerator temperature.

Oxalic acid and theobromine did not have any noticeable effect on the growth of bacteria.

The palatability of the chocolate milks, and especially of those samples

stored at room temperature, was directly comparable with the bacterial counts of the milks.

The conclusion seems to be justified that cocoa powders or chocolate syrups (excepting those flavored with cocoa extracts) added to milk definitely inhibit the growth of bacteria likely to be found in milk, with the exception of *Streptococcus lactis*, and that the tannic substances of the cocoa products are the agents responsible for inhibiting bacterial growth.

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THE EFFECT OF STILBESTROL AND ANTERIOR PITUITARY EXTRACT UPON LACTATION IN GOATS*

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The production of fully developed lactating mammary glands during pregnancy requires a high degree of synchronization between the pituitary, ovaries and placenta. The entire mammary developmental process can be stimulated by the artificial administration of now available natural and synthetic hormones to virgin or castrate animals. The administration of a synthetic estrogen (diethylstilbestrol) which has many physiological properties of the natural estrogens, to animals with intact pituitaries, causes the development of complex mammary duct systems such as are present in the virgin but mature animal (Turner for review, 51). In at least two species, the guinea pig and monkey, estrogens cause the development of complete mammary glands with lobule-alveolar systems such as are present at midpregnancy. But in most species so far studied the addition of progesterone, the corpus luteum hormone, to this estrogen treatment greatly facilitates the growth of the mammary gland and carries the development to completion in animals with intact pituitaries (51).

In addition to their mammary growth-stimulating properties, estrogens have the ability to cause a great increase in the secretion by the anterior pituitary of the lactogenic hormone, which is necessary for the initiation and maintenance of lactation (44, 31, 36). This simulates the condition at normal parturition when estrogenic action becomes dominant over that of progesterone and lactogen secretion pours into the blood and causes the great surge of milk secretion at that time (37, 38). Lactation after parturition comes from fully developed lobule-alveolar glands the growth of which has been stimulated by estrogen and progestin during pregnancy. With the administration of estrogen, however, milk secretion can be induced in less fully developed glands.

The synthesis by English workers of a drug, diethylstilbestrol, which has many of the properties of the natural estrogens and in addition is active on oral administration and can be produced cheaply has stimulated experimentation on the stimulation of growth of the mammary gland and lactation in dairy animals.

Folley *et al.* (17) reported that diethylstilbestrol dipropionate ointment applied to the udders of three virgin goats caused copious lactation. Three virgin heifers responded only slightly (17, 18). Lewis and Turner (29, 30)

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induced lactation in two virgin kids, one of which was a castrate, and in two yearling does given subcutaneous injections of diethylstilbestrol. An aged, sterile cow lactated from the single normal quarter of the udder on diethylstilbestrol treatment (28). Walker and Stanley (52) secured a maximum of 14 and 16 pounds of milk daily from a castrate heifer and a sterile cow after several months' injection of diethylstilbestrol dipropionate plus testosterone propionate. Folley and Young (21) showed that a crude anterior pituitary extract would increase the milk yield of a diethylstilbestrol treated goat or bring into heavy lactation one which had not responded to the estrogen.

Lactation has also been reported from goats due to the action of other estrogens plus anterior pituitary extract. DeFremery (11) reported that percutaneous inunction of the udder with estradiol benzoate caused the development of goat udders of parturient type. Anterior pituitary extract then produced abundant lactation. Trautman and Kirchhof (50) gave orally a total extract of ovary (Oestruzyl) plus an anterior pituitary extract and secured from 480 to 900 ml. of milk daily from several goats.

Anterior pituitary extract alone was reported by Evans (13) to cause considerable lactation in virgin and dry goats and cows whereas Catchpole *et al.* (9) and Asdell (2) had little success with this treatment.

PROCEDURE

During subcutaneous administration of diethylstilbestrol, the chemical was given once daily dissolved in oil. Injections were made in front of or behind the shoulders. For percutaneous administration diethylstilbestrol was dissolved in 70 per cent alcohol. Sufficient carrier was used to cover the udder when released at the dorsal margin and allowed to run over the skin with some manual spreading. The long hairs were clipped from the udder at the beginning of the experiment. The alcohol quickly evaporated. For oral administration diethylstilbestrol was mixed with glucose and the daily dosage added to the grain fed. It appeared to be quite palatable in this form. Hard pellets of diethylstilbestrol dipropionate weighing 100 mg. were implanted subcutaneously behind the shoulder through an incision in the skin.[1]

The anterior pituitary extract used was an initial extract prepared by A. J. Bergman of this laboratory. It contained 4000 I.U. of lactogen per gram, 3000 chick units of thyrotropin, 1000 chick units of gonadotropin, 250 guinea pig units of blood-sugar-raising principle and 100 chick units of adrenotropin as assayed by Mr. Bergman.

The goats in this experiment were on dry feed at all times consisting of alfalfa hay and a mixed grain ration. They were milked twice daily.

[1] The diethylstilbestrol powder was contributed by Merck and Co. and the pellets by the Blue Line Chemical Co., St. Louis.

COMPARISON OF DIFFERENT METHODS OF ADMINISTRATION

We have already reported that subcutaneous injection of diethylstilbestrol was effective in initiating and maintaining lactation in goats (29, 30). This method of administration has perhaps less practical application than others such as percutaneous application to the udder, implantation of pellets

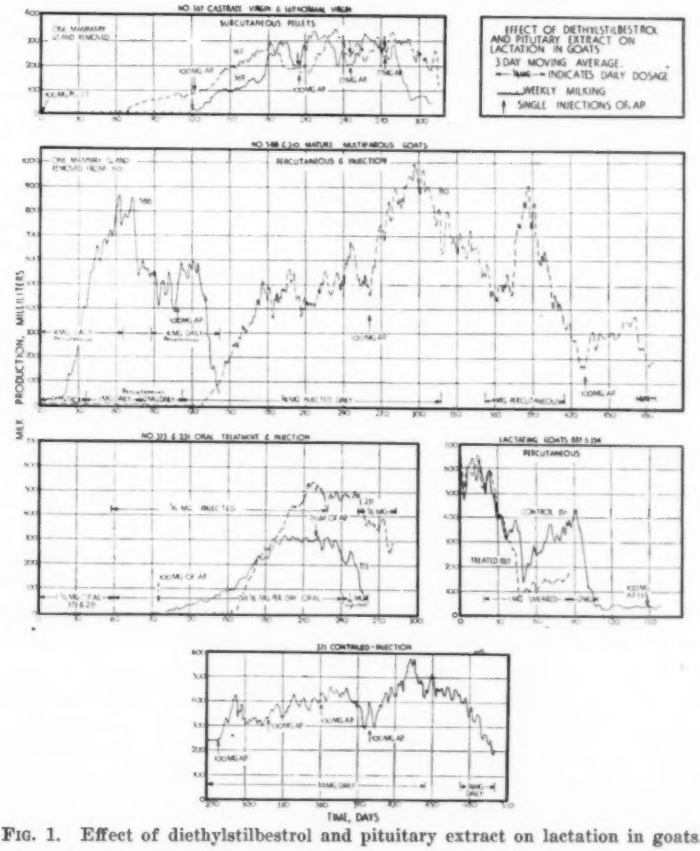

FIG. 1. Effect of diethylstilbestrol and pituitary extract on lactation in goats.

or especially oral treatment. We therefore wished to compare the effectiveness of these different methods of administration in goats.

Administration of hormones by *subcutaneous injection* is probably the most common means used for laboratory animals and the method first used by us in giving diethylstilbestrol to goats. Besides the four goat lactations previously reported, two additional lactations have been secured from goats by injection of diethylstilbestrol. Goat #310, which had failed to respond

to percutaneous application, readily came into production on ¼ mg. per day by injection and reached a production of a liter a day after a single dose of anterior pituitary extract (fig. 1). No. 231, a virgin yearling goat, produced over 500 ml. of milk a day on a similar injection dosage after failing to respond to oral treatment. Also we previously reported eight months of lactation from #371 given the same dosage (29, 30). She continued to lactate for seven more months and with periodic injections of anterior pituitary extract she recovered to her previous peak of production, *i.e.*, 600 ml. (fig. 1).

Administration of diethylstilbestrol by subcutaneous injection appears to be an efficient method of initiating lactation in goats. A very low dosage was required. The labor of treatment could be reduced by giving two or three injections a week instead of daily.

Percutaneous treatment of laboratory animals with estrogens has been shown to be efficient in causing genital changes (53, 10, 42) and mammary gland growth (47, 33, 24). We found that 0.01 mg. of diethylstilbestrol applied daily to the shaved skin would cause extensive mammary gland growth in male rabbits in 30 days (32).

Percutaneous inunction of the udder is a convenient method of administration of diethylstilbestrol. This is the method used by Folley *et al.* (17, 21) who treated the udders of goats three times a week giving a total of 30 mg. We found that 7 and 14 mg. per week were ineffective in a mature dry goat (#310) but 28 mg. promptly brought a second goat (#588) into lactation. Four milligrams daily applied to the udder favorably affected the established lactation of #310 but 10 mg. had little effect on the lactation of a cow (28). One-quarter milligram daily by injection caused copious lactation in the first goat and in several others. According to these results on a dosage basis injection of diethylstilbestrol is much more efficient than percutaneous administration in causing lactation in goats. Eight times our usual injection dosage did not cause lactation while 16 times the subcutaneous dosage was effective.

Oral administration of a drug has considerable advantage over other methods for clinical or practical use in farm animals. In comparison to the subcutaneous dosage diethylstilbestrol has been shown to be comparatively much more active orally on the genital organs and mammary glands than are the natural estrogens in laboratory animals (30, 27, 41) and in primates (35, 23, 7).

Oral administration for other purposes than milk secretion has been shown to require about five times the dosage of diethylstilbestrol as by injection. When we gave this amount (1.25 mg. daily) to three goats (#373, #355, #231) no lactation response was secured for 50 to 123 days. No. 355 gradually began to lactate when raised to 2.5 mg. daily and responded to anterior pituitary extract. However, #375 responded to an anterior pituitary extract injection with lactation when being given only 0.625 mg. per

day (fig. 1). Ten times the injection dosage to a fourth goat (#360) had no effect, but she began to lactate when 5 mg. were given daily. These three goats which finally lactated on oral treatment produced a maximum of about 300 ml. of milk daily. From our previous results we had expected them to exceed 500 ml. Indeed one of these goats (#373) had lactated for six months with a maximum of 550 ml. under diethylstilbestrol injections the previous year. One of the three goats (#231) which did not respond to 5 times the injection dosage reached 575 ml. of milk per day when given ¼ mg. daily injections.

Why these goats did not respond better to oral dosage is uncertain. It may be that diethylstilbestrol is destroyed in the rumen and if placed directly in the reticulum the treatment might have been more effective. These goats may have been more recalcitrant to diethylstilbestrol treatment than usual and would have responded to anterior pituitary extract injection sooner. Folley and Young reported such a case (21). In a trial of the effect of diethylstilbestrol on the chemical enrichment of milk produced by dairy cows Folley *et al.* (19) also found that oral administration did not have the expected effect.

Subcutaneous implantation of pellets is the mode of administration of diethylstilbestrol involving the least labor. The continuous absorption of the hormone makes for more efficient use, and it has been shown to be quite effective (12, 23). MacBryde *et al.* (34) reported that on a dosage basis pellets were 5 to 10 times as effective as injections in castrate women. The effect of 100 mg. pellets lasted for 400 to 800 days. From 0.127 to 0.25 mg. was absorbed daily. We used the dipropionate form in this case which is somewhat more slowly dissolved.

Two virgin yearling goats (#369, #367) each of which had a 100 mg. subcutaneous pellet of diethylstilbestrol dipropionate came into milk production after a long latent period. With the addition of anterior pituitary extract at 25–35-day intervals production rose to 350 ml. (fig. 1). This greatly exceeded the production secured the previous year from these goats when treated by injection. This was in spite of the removal of half of the udder after the previous lactations. It seems probable that the long latent period could have been shortened by the earlier administration of anterior pituitary extract. As will be shown in another report, considerable growth of the mammary glands occurred. It appears probable that implantation of pellets or crystals of diethylstilbestrol is a very efficient method of securing milk production from virgin or dry dairy animals especially in combination with anterior pituitary extract.

EFFECTS OF ANTERIOR PITUITARY EXTRACT INJECTION

Anterior pituitary extracts have been shown to be effective in bringing into lactation laboratory animals with prepared mammary glands (51).

Folley (21) reported a case of a goat which did not come into milk production on diethylstilbestrol administration until anterior pituitary extract had also been given. In addition there have been several reports that anterior pituitary extract injections will increase milk production in lactating cows (4, 20), and women with deficient lactation (26, 45, 25). Sykes *et al.* (49) obtained good results in cows with a fat metabolism extract of anterior pituitary but poor results with a lactogenic extract.

Single injections of anterior pituitary extract seemed to have an activating effect on lactation in diethylstilbestrol-treated goats. Instead of there being a brief rise in production, production tended to improve for some days after the injection. The time from injection to the subsequent decline in production was 62, 43, and 47 days with successive injections in #367. In the case of #369, peaks occurred 67, 18, 10, 7 days after injection. With #310 production did not decline for 37 and 38 days after single injections of anterior pituitary extract. In the case of #371, production continued to rise for 13, 25, 20 and 36 days after anterior pituitary extract injections.

The amount of increase in production from anterior pituitary extract injections was gratifying in most cases. The best percentage increases in the case of goats already producing fairly well were 100 per cent (#367); 110 per cent (#369); 90 per cent (#371) and 150 per cent (#310). Other responses were less. No. 369 responded better to 100 mg. injections than to 77 mg.; 1 mg. per pound of body weight. No. 373 did not respond at all to such an injection.

An initial extract of anterior pituitary proved to be a valuable addition to diethylstilbestrol therapy of goats. We previously reported the use of a more purified lactogenic extract of anterior pituitary under similar conditions (29, 30). Lactogenic extract did not prove to be as effective as an extract with 95 per cent of the inactive protein removed but containing large amounts of other anterior pituitary hormones such as thyrotropin, adrenotropin, blood-sugar-raising principle, gonadotropin and possibly other unassayed factors. This is in agreement with the results of other investigations on the effects of anterior pituitary extracts on milk production (4, 20). Although lactogen is the principal hormone controlling initiation and maintenance of lactation other anterior pituitary factors act synergistically with lactogen in the galactogogic effect (5). Thyrotropin especially, acting through the thyroid gland, causes an increase in metabolism of cells and in quantity of milk production. Since administration of estrogens causes a great increase in lactogen output of the pituitary; lactogen should not have been a limiting factor in these lactations.

Further evidence that the response to anterior pituitary extract observed in this study was not a response primarily to lactogen is provided by a study of the dosages administered. To bring a pseudo-pregnant rabbit into good lactation requires 260 McShan-Turner units of lactogen or 33 McShan-

Turner units per pound body weight given in six daily injections (6). At this rate a 75-pound goat should require 2475 McShan-Turner units in proportion to body weight. This amount of lactogen was contained in 495 mg. of the lactogenic extract used previously or in 614 mg. of initial anterior pituitary extract used in this study. The lactogenic extract was given at the rate of one milligram per pound of body weight per day for 6 to 12 days. The response was almost immediate in three cases and lasted little beyond the period of injections. Averaging the milk production for 10 days before and after these injections the increase in production amounted to 6 and 14 per cent (#373), 19 and 1 per cent (#371). However, when a single 100 mg. injection of initial anterior pituitary extract was given the increase in production was as high as 150 per cent and continued for as long as 30 to 40 days. Thyrotropin has a prolonged effect on metabolism in some cases, but it also affects the heart rate (43). The pulse rate of five of these goats was recorded daily for 48 days after a single injection of 100 mg. of anterior pituitary extract. There was no apparent effect in four cases in which good lactation response was secured. The pulse rate of the fifth goat increased from 93 to 136 beats per minute and remained above 100 for 41 days. This goat did not increase in milk production in response to the anterior pituitary extract injection. Probably the response to initial anterior pituitary extract was due to a combination of the various pituitary factors contained therein.

Administration of estrogen may not increase the production of other pituitary hormones as it does that of lactogen (46, 39). By supplying a whole extract of anterior pituitary, we were increasing the supply of other pituitary hormones needed for increased milk production.

THE EFFECT OF NON-CONTINUOUS ADMINISTRATION OF DIETHYLSTILBESTROL

Walker and Stanley (52) reported that intermittent treatment with diethylstilbestrol dipropionate could be used to cause further increases in milk production in a virgin cow which had been brought into production with injections of the hormone.

We have tried the effect of stopping treatment after a peak of production had been reached and a decline had set in in 9 cases. Stopping treatment generally seemed to have the effect of terminating the decline in production in most cases within one to seven days (#360, #355, #231, #427,[1,2] #371, #373). Production either returned to normal (#360, #355, #231) or increased somewhat for 2 to 10 days (#588, #427,[1,2] #371, #373). A decline again set in after 10 to 25 days (#588, #427,[1,2] #371, #373). No effect of stopping treatment was observed in one case (#310[1]). Production continued to decline for 24 days in another (#310[2]).

When treatment was resumed after 22 to 72 days the results were variable. Production tended to level off after a latent period of 1 to 12 days

(#231, #427, #310[1, 2]) and then dropped in three cases (#231, #310[2], #427), increased for 2 days then slowly declined (#355), dropped and then recovered (#360, #588) or had no effect (#371).

A decline in production from diethylstilbestrol treatment may occur after a time due perhaps to the accumulation of excess hormone or to loss of its effectiveness. If treatment is then terminated production may plateau or even recover somewhat and then again begin to decline. Resumption of daily treatment at this time was not usually successful in holding up production for long. Injection of anterior pituitary extract was much more efficacious.

EFFECT OF DIETHYLSTILBESTROL ON PARTURIENT GOATS

It was considered that the effect of diethylstilbestrol on normal parturient goats already in milk should be investigated. If this chemical were capable of increasing the milk production of such dairy animals, it would be of considerable practical advantage. There are many reports, however, that estrogens will inhibit lactation as well as reports to the contrary (see 36 for review). Folley (14) reported that estradiol benzoate and estrone caused a reduction of 20 per cent in milk production of cows but the milk was richer in fat and solids not fat. Diethylstilbestrol implanted subcutaneously or injected into lactating cows did not cause any reduction in milk volume but increased the proportion of solids in other cases (15, 48).

Whether or not estrogens cause a reduction of milk production in lactating animals is perhaps a question of dosage. High dosage will certainly inhibit lactation. We were interested in learning what dosages of diethylstilbestrol similar to those which caused lactation in dry goats would do to milk production in normal parturient goats. We have already reported the inconclusive results secured in two such cases (29, 30).

Two additional parous goats which were already in milk were treated by percutaneous application of diethylstilbestrol. No. 351 served as the control at first and #837 was given 1 mg. per day. Both goats declined rapidly in production due probably to a spell of cold weather (November). The control goat then recovered to 440 ml. while the production of the treated goat only rose to 180 ml. during the next month. The control goat (#351) was then given twice the dosage of diethylstilbestrol. Production promptly plunged from 430 ml. to 40 ml. in 15 days where it was maintained during the next 50 days in spite of termination of treatment. An injection of 100 mg. of anterior pituitary extract had no effect.

The administration of diethylstilbestrol to parturient goats was at least of no obvious advantage on volume of milk production from mammary glands already in secretion. The early decline in production of #837 when diethylstilbestrol was applied to the udder would have looked like a clear case of suppression of lactation if the control goat had not declined similarly.

The poor recovery of #837 may or may not have been due to the treatment. The response of #351 might be considered a clear-cut case of suppression of lactation except that the dosage used (2 mg.) had proven inadequate for the initiation of lactation in a dry goat (#310). Twice that dosage readily brought a second goat (#588) into heavy lactation and when used later on #310 in the declining phase of her lactation it caused a precipitous, though temporary, rise. That a certain dosage of diethylstilbestrol would bring dry goats into long sustained lactation and yet would cause normal parturient goats to dry up does not appear rational in the present state of our knowledge.

EFFECT OF DIETHYLSTILBESTROL TREATMENT ON THE FUTURE BREEDING HISTORY

The effect of diethylstilbestrol on the subsequent reproductive ability of treated animals is of considerable practical interest. If no permanent deleterious influence is to be expected, diethylstilbestrol might be useful in such cases as when goats, which have a seasonal breeding period, have failed to conceive. Thus, instead of losing a year's production, these open goats could be made to pay for their keep. There would also be less prejudice against diethylstilbestrol treatment of cows which have failed to breed but which might eventually recover. According to Allen *et al.* (1), many descriptions of ovaries following estrogen treatment have reported inhibiting or depressing effects on follicular development. This probably occurs through a depressing effect on the follicle-stimulating hormone of the anterior pituitary. If the hormone treatment is not too severe, the depression may be temporary and the ovaries may recover fully soon after treatment is stopped.

Most of the diethylstilbestrol treated goats in this experiment were under continuous treatment for long periods of time. On the other hand, the amount of chemical administered was kept as low as was practical. Under these conditions most of the goats showed cyclic estrus; that is, there were periods during which the characteristic signs of estrus were especially prominent and they would accept the buck more readily. At these times milk production tended to decline temporarily. Similar cyclic response to continuous estrogenic treatment has been reported in laboratory animals (8).

The ovaries of these diethylstilbestrol treated goats were examined at autopsy after termination of the treatments. The ovaries from #371, which was under almost continuous diethylstilbestrol treatment for 428 days, were definitely abnormal. They appeared to have degenerated until normal ovarian tissue was absent. On the other hand, the ovaries from #310 were normal in appearance at autopsy weighing 5.46 gm. and containing several large follicles. No. 310 had had a rest period of 72 days without treatment shortly before autopsy which might have allowed some recovery to occur. No. 371 also had a 29-day rest period. The ovaries of #231, #373 and

#355 were in an essentially normal although quiescent condition while one ovary of #360 had a corpus luteum. No. 369, which had had a series of 96 daily injections and then a subcutaneous pellet for 331 days, a total of 427 days, had ovaries which weighed 12.7 g. and contained several 2 cm. follicles. These ovaries would undoubtedly have recovered promptly on cessation of treatment.

Two normal parturient goats (#370, #443) had been given daily injections of diethylstilbestrol for 20 and 107 days respectively. They were rested 49 and 157 days and then were given 5 to 6 ml. injections of pregnant-mares-serum extract (Gonadin[2]) at 16-day intervals. No. 370 bred after each of three treatments but apparently did not conceive. No. 443 conceived after the second treatment (April) and bore a rather small but otherwise normal kid.

Thus in only one case after 15 months treatment with diethylstilbestrol was an abnormal condition of the ovaries found. The ovaries of three goats were quiescent with very small follicles. One goat had large follicles; a second had normal sized follicles; a third had a corpus luteum indicating that ovulation must have occurred. That such ovaries were capable of recovering their normal function is shown by the response of two goats to gonadin treatment.

THE EFFECT OF DIETHYLSTILBESTROL IN PREGNANT GOATS

There have been many reports of the termination of pregnancy by administration of estrogens (1). This is a dosage problem for small dosages may not affect pregnancy. The greater the interval between fertilization and initial treatment the larger the amount of estrogen tolerated. Abortion has been reported in sheep within 1 to 8 days of the injection of 20,000 international benzoate units (3). Folley and Scott-Watson (16) reported abortion in two of four cows in late pregnancy after 13 inunctions of the udder with 2.9 gms. of diethylstilbestrol dipropionate in 31 days.

Our results with two goats do not substantiate this. The udder of goat #443 was smeared with 4 mg. per day of diethylstilbestrol from the 63rd to the 136th day of pregnancy and during the last 6 days. A normal kid was produced on the 149th day of pregnancy. A second goat (#360) was fed 1.25 mg. a day of diethylstilbestrol for the last 73 days of pregnancy and kidded normally.

The dosage of diethylstilbestrol administered by Folley and Scott-Watson was 1.5 times that which we gave goat #443 on a body weight basis (assuming 1000-lb. cows). Possibly if the dosage to these goats had been several times greater, abortion would have resulted. However, dosages high enough to initiate milk secretion in dry goats did not cause abortion.

[2] Contributed by the Cutter Laboratories.

THE EFFECT OF DIETHYLSTILBESTROL ON THE BODY WEIGHT OF GOATS

An adverse effect of diethylstilbestrol on body weight of laboratory animals has been reported (40). Six to 10 mg. subcutaneous pellets caused a daily loss in body weight of 0.3 to 3.6 gm. in male and female rats (22). As low a dosage as 2 to 3 mg. a day caused a slight but definite inhibition of the growth of male rats (41).

The body weights were available for nine of these goats taken at the beginning of long periods of treatment with diethylstilbestrol and again at termination (table 1). The periods of treatment were from 9.5 to 17 months. Seven young female goats and two males gained from 20 to 43 pounds during the treatment and were of essentially normal size for their age. The goats were in good health during the entire time. They appeared thrifty and ate well. One mature goat (#310) was thin for some time during her treatment and would not consume sufficient grain to gain weight. That this was due to the diethylstilbestrol treatment is doubtful. The two males listed had 200 and 277 mg. pellets of diethylstilbestrol dipropionate implanted subcutaneously.

TABLE 1

Gain in body weight of goats during continuous diethylstilbestrol treatment

Number	Date	Body weight	Termination		Length of treatment	Gain in body weight
			Date	Body weight		
		lbs.		*lbs.*	*mos.*	*lbs.*
231	11/40	48	9/41	72	10.7	24
360	3/41	62	10/41	82	6.7	20
355	12/40	38	10/41	64	9.5	26
373	4/40	48	10/41	71	15.3	23
371	7/40	75	9/41	98	15.0	23
367	7/40	47	10/41	90	14.3	43
369	7/40	42	9/41	78	13.3	36
43 ♂	11/40	32	9/41	66	11.0	34
833 ♂ Castrate	4/40	86	9/41	113	17.0	25

SUMMARY

Administration of diethylstilbestrol by subcutaneous injection, orally, by inunction or diethylstilbestrol dipropionate by implantation of pellets caused copious and prolonged lactation in virgin and dry goats. This is significant because of the ability of estrogens to increase the anterior pituitary lactogenic output. One-quarter milligram per day was sufficient by injection. Oral administration appeared to be least effective up to 5 mg. per day or 20 times the subcutaneous dose. Inunction of the udder required 4 mg. daily or 16 times the subcutaneous dose. Anterior pituitary extract was effective in augmenting the production caused by diethylstil-

bestrol by supplementing the increased lactogen output with other anterior pituitary factors or in initiating lactation in diethylstilbestrol-treated goats. Stopping daily treatment was sometimes effective in improving production for a time but restarting treatment had little effect. Hormone treatment of normal parturient goats was not beneficial on volume of production. Treatment during the last half of pregnancy had no deleterious effect in two cases. Ovaries were essentially normal after long periods of treatment with one exception. Two goats were bred out of season after termination of diethylstilbestrol injections. One conceived and bore a kid. No adverse effect on growth or health of the goats from diethylstilbestrol treatment was observed.

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CONTENTS

Bacteriology
Breeding
Butter
Cheese
Chemistry
Concentrated and dry milk; by-products
Diseases
Feeds and feeding
Food value of dairy products
Herd management
Ice cream
Milk
Miscellaneous
Physiology

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Ice Cream Review
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Journal of Industrial and Engineering Chemistry
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United States Department of Agriculture

ABSTRACTS OF LITERATURE

BOOK REVIEW

584. Chemistry and Physiology of the Vitamins. H. R. ROSENBERG. May, 1942. Interscience Publishers, Inc., 215 Fourth Ave., New York. 674 pages, 25 figures; $12.00.

Recognizing the need for a comprehensive review of the chemistry and physiology of the vitamins, Dr. Rosenberg has presented in this book a concise, up-to-date and systematic review of all topics of vitamin research which have contributed to the rapid development and advancement in the knowledge of this field.

The opening chapter is a discussion of vitamins in general which includes definitions, historical development, nomenclature, list of vitamins and a general discussion of the topics which are followed systematically in every chapter on the individual vitamins.

In the chapters on the individual vitamins, there appears a concise review of nomenclature, historical development and occurrence. The discussions on the chemistry include methods of isolation, proof of chemical constitution and synthesis including space formulas for the various steps and industrial methods of preparation. There are special paragraphs on the biogenesis and specificity of vitamin action. The determinations of vitamins are subdivided into physical, chemical, biochemical and biological methods and are followed by a discussion of vitamin standards. The physiological treatment includes relationships in plant and animal physiology, the latter being subdivided into the metabolism of vitamins, the physiological action, mechanism of vitamin action and the relation of vitamins to each other, to hormones and minerals. The pathological aspect treats with avitaminosis, hypovitaminosis, hypervitaminosis and a special section on clinical tests. Finally the vitamin requirements are presented.

Following the chapters on the individual vitamins, there is a brief discussion of 17 non-identified vitamins. In the appendix is classified a group of essential compounds which are called vitagens. These include essential fatty acids, amino acids, carbohydrates, choline and related compounds and organic sulphur containing compounds.

In the patent index there are listed more than 1200 United States, British, German and French patents which are arranged chronologically according to subject matter. The essential data of each patent is given together with a brief abstract.

This book should appeal both to those engaged in vitamin research and to those who wish to be informed on this subject. It is well written and conveniently arranged for use as a reference text, with the bibliography

readily available as footnotes. The reviews include literature through 1941 and even into 1942. S. M. Hauge

585. A Symposium on Respiratory Enzymes. University of Wisconsin Press, Madison, Wis. 1942. 281 pages; $3.00.

This book deals with the fundamental nature of those enzymes and enzyme systems that are closely connected with the action of vitamins. Each part of the book is written by an international authority in his field. Recent findings are discussed in detail and interpretations of most developments are made. The discussions on Phosphorylation, Respiration, and Hydrogen Transport which apply the fundamental findings to important, specific problems are especially informative and interesting.

The divisions on Intermediate Carbohydrate Metabolism by Meyerhof; Oxidative Mechanisms in Animal Tissue by Ball; Oxidases, Peroxidases and Catalase by Stern; Flavoproteins by Hogness; Cytochromes by Stotz; and Phosphorylation of Carbohydrates by Cori are especially well written. The sections on Pasteur Effect by Lipman; Nicotinamide Nucleotide Enzymes by Schlenk; Metabolic Cycles and Decarboxylation by E. A. Evans, Jr.; and Transamination by Cohen give information that is very timely.

All sections of the book carry good bibliographies and the book as a whole should serve as an excellent reference for individuals that are working directly in the field of respiratory enzymes. D. M. Doty

586. War Gases. Morris B. Jacobs. 1942. Interscience Publishers, Inc., 215 Fourth Ave., New York. 180 pages, illustrated; $3.00.

This book deals with the following general subjects in the field of war gases: 1. Chemical nature, physical characteristics and physiological response of the war gases. 2. Effect of war gases on materials, food and water and methods of obtaining samples of these for analysis. 3. Methods of detection of and analysis for toxic gases in foods, water, air and other materials. 4. Decontamination of various materials polluted by such gases.

The author stresses the importance of a complete knowledge of the problem, to intelligently and actively cope with any possible gas attacks in this country regardless of how remote they may appear at the present time. The information presented is of interest primarily to specialists in this field such as the war gas chemist, gas identification, decontamination and public health officers and air raid wardens.

Manufacturers and distributors of dairy products might well read those sections of the book dealing with effect of various gases on foods as well as discussions on removal of samples for analysis and methods of decontaminating after gas attacks. The author also states "The best way to obviate the necessity for salvaging foods after gas attacks is to protect them from contamination before the attacks." As is also emphasized, adequate protec-

tion must be based on a knowledge of the properties of the gases. Methods of protection must be known and put into practice before any possibility of attack exists in order to be sufficiently adequate to meet all emergencies.

This book is well written throughout. Many details regarding precautions and procedures are emphasized to the point of repetition. This is undoubtedly desirable from the standpoint of certain individuals not too familiar with all aspects of the subject but who yet are responsible for coping with any possible emergency. One fact very well brought out in the discussions is the complexity of the problems involved and the necessity for supervision of all gas defense efforts by experts in the field of gas warfare.

A publication of this size naturally could not include all of the useful information on the subject. The author therefore has preceded each chapter with general references. Frequent references are made in the text to other published investigations from which the author has drawn his information. The reader can thus obtain more detailed information on practically any phase of this subject. Complete author and subject indices also enhance the value of the book. P.R.E.

587. Annual Review of Biochemistry, Volume XI. Annual Reviews, Inc., Stanford Univ. P. O., California. 736 pages; $5.00.

The Annual Review of Biochemistry, as once before cited in the JOURNAL OF DAIRY SCIENCE book review, is an assembled group of reviews on selected biochemical subjects written upon invitation, by competent authorities. The Volume XI contains 28 sections and includes reference to some 3929 papers. A significant number of the reviews in this new issue are of real interest to those engaged in dairy products research. The Annual Review is written in technical research rather than popular terminology.

In X-Ray Studies of the Structure of Compounds of Biochemical Interest is presented a review of the background of knowledge concerning the details and principles of molecular and crystal architecture and experimental methods. Illustrations are employed to explain principles. Reference is made to studies on fibrous, corpuscular and virus proteins, carbohydrates and sterols, porphines and glycerides. The section on the Chemistry of the Acyclic Constituents of Natural Fats and Oils contains review of methods recently developed for the separation and identification of the individual and component fatty acids. Excellent discussion is presented on distribution and composition of acids, glycerides and phospholipids in vegetable, marine and animal fats. A very complete discussion is presented on the Chemistry of the Proteins and Amino Acids, and includes the size and shape of protein molecules, the number and configuration of the electrically charged groups in amino acids, peptides and proteins, and implication of the data for biochemical studies. The Metabolism of Proteins and Amino Acids is excellently reviewed. In two excellent chapters are considered the

developments in the fields of the water- and fat-soluble vitamins. Since an immense flow of investigative work in the vitamin field is taking place, orientation and evaluation of the information, inferentially applicable to milk, is useful. Vitamins discussed in the first of the two sections include thiamine, nicotinic acid and nicotinamide, riboflavin, pyridoxin, pantothenic acid, choline, biotin, inositol, p-aminobenzoic acid, ascorbic acid, citrin; in the second, vitamins A, D, E, and K. The section on Calcium and Phosphorous Metabolism: Clinical Aspects, presents pertinent review on the relative availability of the calcium of milk, and on the mode of action of certain antirachitic factors. In the chapter on Biochemical and Nutritional Studies in Relation to the Teeth is included discussion on the significance of calcium and vitamin D in occurrence of dental caries. The nutritional demands of fowl is reviewed in Avian Biochemistry and is of value of interpreting better the nutritional adequacy in rations of milk by-products. In addition to the above chapters are: Biological Oxidations and Reductions; Hydrolytic Non-Proteolytic Enzymes; Chemistry of the Steroids, of the Hormones, of Visual Substances, of Muscle; Chemistry and Metabolism of Compounds of Phosphorous; Carbohydrate, and Fat, Metabolism; Lignin; Animal Pigments; Alkaloids; Mineral Nutrition of Plants; Plant Tissue Cultures, Immuno Chemistry, and Microbiology (including anti-bactericidal agents). The volume contains a subject and author index. Each section is provided with references cited in the review. K.G.W.

BACTERIOLOGY

588. Glutamine and Glutamic Acid as Growth Factors for Lactic Acid Bacteria. MAXWELL A. POLLACK AND MANFRED LINDNER, Dept. Chem., Univ. Tex., Austin. Jour. Biol. Chem., *143*, No. 3: 655. 1942.

Nine lactic acid-producing bacteria are shown to require either glutamine or glutamic acid for growth. None of the bacteria tested is capable of producing glutamine from glucose and ammonium salts. V.C.S.

589. Routine Bacteriological Examination of Milk Cartons. W. A. HOY, Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 48. 1941.

Of 1654 waxed paper pint milk cartons examined over a period of 6 years, 97% contained less than 200 recoverable bacteria per carton and 86% were virtually sterile. Of 20 cartons that gave counts over 600 (more than 1 per ml. of capacity), 16 were found to be crushed when they were unpacked. Milk souring organisms were found only occasionally. The results of the examinations show that waxed paper milk containers can be made and distributed to dairies in a satisfactory hygienic condition, and ready for immediate use. M.W.Y.

590. The Examination of Raw Milk by the Methylene Blue and Resazurin Tests and by the Plate Count. CONSTANCE HIGGINBOTTOM, Hannah Dairy Res. Inst. Proc. Soc. Agr. Bacteriologists (Eng.), 32. 1941.

Nearly 1,000 samples of raw milk from individual farms were examined by the plate count, methylene blue reduction test, and resazurin test. The samples were examined immediately on arrival at the laboratory and again after storage overnight at 40° F., and 60° F. On identically treated samples the correlation coefficient between the plate count and the methylene blue reduction test was high, *i.e.*, 0.84, 0.85, 0.90, and 0.73 (negative values) respectively. A very close agreement was obtained between the time taken to reduce methylene blue and to reach the vivid pink stage (Lovibond disc No. 1) with resazurin. M.W.Y.

591. The Micrococci of Milk. T. GIBSON AND Y. ABDEL-MALEK, Col. Agr., Edinburgh. Proc. Soc. Agr. Bacteriologists (Eng.), 31. 1941.

Pasteurization at 63° C. for 30 minutes of samples of certified milk and aseptically drawn milk destroyed all micrococci while those in milk of indifferent quality frequently survived the treatment. It is concluded that the cow's udder is not a source of the heat-resistant species. M.W.Y.

592. Resazurin Reduction of Fresh and Stored Udder Milk. S. B. THOMAS, Univ. Col. Wales, Aberystwyth. Proc. Soc. Agr. Bacteriologists (Eng.), 34. 1941.

Forty-four aseptically drawn samples of quarter milk from normal and latent mastitis infected udders were examined within one hour of milking, and portions of the same milk held at 5° C. and 15° C. were examined after 24 hours. Holding the milk for 24 hours resulted in considerable loss of resazurin-reducing activity. There was no significant difference in the reduction times of the portions held at the two temperatures. M.W.Y.

593. Cryophilic Bacteria as a Cause of Milk Samples Failing in the Methylene Blue Test. C. S. MORRIS, Scale-Hayne Agr. Col., Newton Abbot, Devon. Proc. Soc. Agr. Bacteriologists (Eng.), 33. 1941.

Samples of morning milk from a certain area failed to pass the methylene blue test after being held overnight in a refrigerator kept at 4° C. *Pseudomonas* types were isolated that grew rapidly in milk held at 4° C. Water supplies on some of the farms contained these *Pseudomonas* types and equipment became contaminated from these supplies. M.W.Y.

594. A Note on the Lipase of Some Lactic Acid Organisms. J. WOLF, Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 21. 1941.

Strains of *Lactobacillus casei, L. acidophilus, L. Lactis, L. delbruckii, L. helveticum, Streptococcus cremoris, S. thermophilus, S. mastitidis, S. mucosus, S. liquefaciens,* and *S. salivarius* were tested for production of fatty acids from butter fat to determine whether they are associated with production of flavor in cheese, where butyric acid and other fatty acids are known to occur. The organisms were grown in a peptone, Lemco, yeast extract, K_2HPO_4 medium (pH 6.9), to which 0.15% agar was added. 0.75% butter fat was added to half the medium. Incubation was at 38° C. and flasks were shaken daily for 7 months. None of the organisms produced fatty acids from the fat.

The possibility of an intracellular lipase was studied using *L. casei.* To digest the cell wall with proteolytic enzymes, a culture of *L. casei* was grown for 6 days in 1 liter of yeast dextrose broth. After centrifuging and washing the suspension aseptically, it was made up to 100 ml. and then divided into two 50-ml. portions. To one was added 2 ml. of a 5% pepsin solution and to the other 2 ml. of 5% trypsin, both of which had been passed through a Seitz filter. The trypsin suspension was brought to pH 8 and the pepsin one to pH 3 and incubated at 37° C. for 24 hours. 10 ml. of each of the digested-bacterial suspensions were placed in flasks and 30 ml. of the butter fat medium used previously were added as substrate. Controls containing the enzymes but no bacterial suspension were also included. The flasks were incubated at 28° C. for 2 months and were shaken daily. After examining each for sterility, 20 ml. from each flask were steam distilled in duplicate. The titrations of 100 ml. of distillate with N/100 NaOH from the tryptic bacterial suspension were 2.00 ml. and 4.80 ml. compared to 1.10 ml. and 1.05 ml. for trypsin only. A Duclaux distillation on the distillate from the tryptic bacterial suspension revealed that over 85% of the acid was butyric. *Lactobacillus casei* contains a lipase or butyrinase which is liberated on disruption of the cell. It is possible that the reason why butyric acid does not appear in cheddar cheese until it is mature is that the butyrinase is liberated at a late stage in ripening. M.W.Y.

595. Resazurin Reduction as an Index of Milk Can Sterility. S. B. THOMAS, Univ. Col. Wales. Proc. Soc. Agr. Bacteriologists (Eng.), 47. 1941.

A series of 154 twelve gallon cans were picked at random after steaming at four creameries, held at atmospheric temperature for 24 hours and rinsed with 100 ml. of quarter strength Ringer's solution. Five ml. of rinsing solution was added to 5 ml. of sterile milk, mixed and incubated for 18 hours at 15° C. The resazurin test was then made. A comparison showed that 90% of the cans with colony counts above 100 per ml. of can capacity reduced resazurin within 1 hour whereas 97% of the rinsings taking over 5 hours to reduce resazurin to the pink stage had colony counts of 10 and under per ml. of can capacity. M.W.Y.

BREEDING

596. Dairy Cattle Breeds. A. B. NYSTROM. U. S. D. A. Farmers' Bul. 1443. Feb., 1942.

This most recent revision of a familiar publication differs from its predecessors of 1935 and 1938 only in bringing up-to-date the number of cows of the different breeds in this country by sections; the milk and butter fat records of the ten highest producers in the United States of each of six breeds of dairy cattle; and in an increased amount of space given to the requirements for recording cows and bulls in the American Dairy Cattle Club.

J.G.A.

CHEESE

597. A Note on the Bacteriology and Chemistry of Portuguese Sheep's Milk Cheese. E. R. HISCOX, S. J. ROWLAND, J. WOLF, AND M. M. JACOB. Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 24–26. 1941.

Examination of 8 cheese varying from 77 to 110 days of age showed that the ripening of Serra cheese like cheddar depends largely on the homofermentative lactobacilli. The volatile acids and the products of the nitrogen breakdown also resembled those of a cheddar of approximately the same age.

M.W.Y.

598. Some Observations on Commercial Starters. J. HARRISON AND J. Z. WOLF, Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 19–20. 1941.

About 140 samples of starters were examined to determine the cause of fluctuations in acid production. Over 100 cultures behaved normally. Young, 18-hour milk cultures were tested in separated milk using 1% inoculum and incubating at 30° C. Two lots of milk were inoculated, one being the control and the other having two drops of whey added. Titrations of 10-ml. portions were made after 2- and 7-hour incubation. Over 60% of the normal starters produced less than 0.35% lactic acid in the 5-hour period under observation. Most of these "sluggish" starters originated from creameries where raw milk was used for cheese making. When the "sluggish" starters stood at room temperature overnight, after the 7-hour titration, and were titrated in the morning according to the usual practice, the acidity was normal. This titration is therefore not indicative of any abnormality which may occur in the starter.

When the addition of whey had an inhibiting effect upon acid production,

only in six cases where the decrease was greater than 0.1% acid was it possible to demonstrate the presence of bacteriophage.

Although bacteriophage might be responsible for many cases of "slowness" where raw milk is used together with a "sluggish" starter, "slowness" may be due to a sudden change in the milk flora or the introduction of milk deficient in acid-producing organisms. M.W.Y.

599. The Problem of Bacteriophage in Cheese Starters. L. J. MEANWELL, United Dairies Res. Lab., London. Proc. Soc. Agr. Bacteriologists (Eng.), 16–18. 1941.

Failure of mixed-strain as well as single-strain starters was frequently due to bacteriophage infection. Bacteriophage was demonstrated by less acid-producing power when 0.1% "slow" whey was added to a 1% inoculation of that culture in sterilized milk. With normal healthy cultures, after six hours incubation at 30° C., there was little or no difference between the control tube and the tube containing 0.1 per cent "slow" whey, but with the susceptible culture an appreciable drop in acidity was found.

Until some system has been devised whereby bulk starters can be completely protected from phage infection, multiple-strain rather than single-strain starters should be used. Phage infection is frequently air-borne and it may be desirable to use a building entirely separated from the cheese-making process for the preparation of starter. M.W.Y.

600. The Evolution of Gases During Cheese Ripening. E. R. HISCOX, Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 23. 1941.

The quantity of CO_2 produced by cheddar and stilton cheese corresponded with the periods of rapid growth, first of the streptococci and later of the lactobacilli. With the growth of the mold in stilton a third increase of CO_2 was noted. The development of CO_2 appears to be largely a direct result of bacterial or mold action. M.W.Y.

CHEMISTRY

601. Color Tests for the Detection of Alpha-Dicarbonyl Compounds Formed in the Autoxidation of Fats. EDWARD A. PRILL, McArdle Memorial Lab., Univ. Wis., Madison. Oil and Soap, *19*, No. 6: 107. 1942.

The tests developed by the author provide definite evidence for the occurrence in autoxidized unsaturated fats of compounds containing either the alpha-diketo (C–CO–CO–C) or the alpha-ketoaldehyde (C–CO–CO–H) group. The presence of alpha-dicarbonyl compounds was found in autoxidized ethyl linoleate, corn oil, cottonseed oil, olive oil and lard. Peroxides

in autoxidized fatty materials could be destroyed by treatment with ferrous chloride without destruction of alpha-dicarbonyl compounds. V.C.S.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

602. Protein-aldehyde Plastics. Combination of Formaldehyde with Acid Casein and with Rennet Casein. D. C. CARPENTER AND F. E. LOVELACE, N. Y. Agr. Expt. Sta., Geneva. Jour. Ind. Eng. Chem., Ind. Ed., *34*, No. 6: 759. June, 1942.

Forms of casein-aldehyde plastic have been manufactured for many years, much of it reaching markets in this country in the form of buttons and buckles. In the manufacture of the plastic the casein is hardened by formaldehyde and it is this chemical reaction with which the authors are primarily concerned. Acid and rennet casein are different from each other in the binding of formaldehyde and their combining ratios are established over a concentration range up to 6.63% formaldehyde. The general law applicable to both types of casein, relating bound formaldehyde to total formaldehyde employed, is shown to be the adsorption law, $x = KC^n$, in the concentration range investigated. About 30% more aldehyde appears to be bound than can be accounted for on the basis of combining with α-amino, side chain amino and amide groups. Possible types of structure are discussed and models are given. B.H.W.

DISEASE

603. Cystic Pituitary in Young Cattle with Vitamin A Deficiency. L. L. MADSEN, S. R. HALL, AND H. T. CONVERSE, Bureaus of Animal Inds. and Dairy Inds., U. S. D. A., Beltsville, Md. Jour. Nutr., *24*, No. 1: 15. July, 1942.

In a series of 15 animals of the beef and dairy breeds, varying in age from one day from birth to 787 days of age, which had either vitamin A deficiency at death or a previous history of vitamin A deficiency, 13 had cystic pituitary glands. The cysts occurred either in the residual lumen or within the posterior lobe, often causing compression of the gland and injury to the glandular parenchyma.

The high incidence of cystic pituitary glands in vitamin A deficient cattle suggests that this is a part of the pathology of vitamin A deficiency. C.F.H.

604. Report of the Committee on Communicable Diseases Affecting Man. PAUL B. BROOKS, Internatl. Assoc. Milk. Sanit. Jour. Milk Technol., *5*, No. 3: 141. May–June, 1942.

The latest report available is for 1939. During this year the U. S.

Public Health Service reported a total of 27 outbreaks were attributed to fluid milk and cream and 8 to milk products. The committee with one exception omitted "one outbreak of undulant fever; two in which neither the name of the disease nor the means by which the milk was supposed to have been contaminated were stated; one bearing a notation: 'Milk or cream suspected'; and a so-called 'outbreak' consisting of one case of gastroenteritis."

Of the 27 outbreaks attributed to fluid milk and cream 4 were typhoid fever; one paratyphoid; 9 scarlet fever and septic sore throat together; 2 bacillary dysentery; and 11 gastroenteritis (including food poisoning).

Of the 8 outbreaks attributed to milk products 6 were of gastroenteritis (including food poisoning), one typhoid and one paratyphoid fever.

Results are also given for the five-year period 1935–1939, inclusive. During this period the total number of outbreaks traced to fluid milk and cream was 175. The committee omitting several "outbreaks" of one case each as they did for 1939. Of the 175 outbreaks scarlet fever and septic sore throat accounted for 68; typhoid fever 62; gastroenteritis 36; bacillary dysentery 5; paratyphoid 3; and diphtheria one.

During this period 25 outbreaks were attributed to milk products. (Three being omitted, one an "outbreak" of only one case and two in which ice cream was reported as suspected, but without explanation.) Of the 25 outbreaks, 22 were of gastroenteritis, one of septic sore throat, one typhoid fever and one paratyphoid fever. Thirteen were charged to ice cream, eight to cheese, two to cottage or cream cheese and two to buttermilk.

A study made of the outbreaks due to fluid milk and cream reported in 1937, 1938, and 1939 with reference to the size of the communities in which the outbreak occurred showed there were a total of 95 outbreaks. Of these 82% occurred in communities of 25,000 or less. L.H.B.

605. The Eradication of Streptococcic Mastitis by Treatment with Tyrothricin. F. E. MARTIN, W. Chester, Pa. Amer. Vet. Med. Assn. Jour., *101*, No. 784: 231. July, 1942.

With an average of 2.3 treatments per quarter, 117 of 130 infected quarters found in 49 of 71 cows were freed of infection. Twelve of the 49 were not completely freed of the infection, while seven cows "with more or less permanent defects of the natural barriers to infection" became reinfected during the winter. No reinfection occurred when cows were on continuous pasture.

Diagnosis was made from smears of incubated samples. Segregation was not practiced nor were precautions taken against spreading infection to healthy cows. Treatment consisted of injecting 20 cc. of a 50% emulsion of mineral oil containing 1.5 mg. of tyrothricin per cc. into the quarter imme-

diately after milking and removing it at the subsequent milking. Infected quarters were examined immediately after treatment and treatment repeated until at least two negative tests at bi-weekly intervals were obtained. Cows were treated in all stages of lactation. S.A.F.

606. Field and Laboratory Observations on Mastitis. P. M. F. SHATTOCK, Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 43–44. 1941.

An attempt was made to eradicate *Streptococcus agalactiae* from two herds of 89 cows. Difficulty in controlling the disease at first was partly due to the high incidence of 21% infection in animals in their first lactation. A further factor was the choice of bacteriological technique, since infections detectable only by enrichment methods may be present for many months before detected on Edward's medium. After the herds became virtually free from *S. agalactiae* infection, high proportions of both hemolytic and non-hemolytic staphylococci were found in many cases. The average percentage of cows excreting non-hemolytic staphylococci in Herd A was 19% and in Herd B 38% while non-hemolytic staphylococci were excreted by 9% and 40% respectively. Subclinical infection with hemolytic staphylococci may give rise to very appreciable alteration in the chemical composition of the milk. Staphylococci may be an unsuspected factor in causing milk to fall below the legal chemical standards. M.W.Y.

FEEDS AND FEEDING

607. Hay Crop Silage. N. N. ALLEN AND J. B. FITCH. Minn. Agr. Expt. Sta. Bul. 360. May, 1942.

A popular bulletin, giving an account of the work done with grass silage at the Minnesota station, setting forth the advantages and disadvantages of this system of storing crops, and containing numerous recommendations and suggestions for the making of grass silage. J.G.A.

608. Feeding Grass Silage. C. B. BENDER. N. J. Agr. Expt. Sta. Bul. 695. May, 1942.

A popular bulletin outlining the advantages of cropping systems which include grass silage and giving results of some of the more recent feeding trials with this type of silage at the New Jersey station. Points stressed are the need for accustoming farmers and cows to this comparatively new type of roughage, the economy of feeding it, and its favorable effect on milk flavor. J.G.A.

FOOD VALUE OF DAIRY PRODUCTS

609. Composition and Thiamin and Riboflavin Content of Defatted Milk

Solids. C. M. O'Malley and E. J. Baldi, Amer. Dry Milk Inst., Inc., Chicago, Ill. Jour. Milk Technol., *5*, No. 3: 138. May–June, 1942.

Thirty-two samples of defatted milk solids from 13 states representing the major milk-producing areas were analyzed during the spring of 1941.

The riboflavin and thiamin content of the samples tested were quite uniform, and there was no apparent relationship between the composition and vitamin content; nor did the geographic origin of the samples show any relationship.

The mean thiamin content of all samples was 3.57 micrograms per gram. The mean riboflavin content was 20.93 micrograms per gram. L.H.B.

610. Nutritional Requirements of Children. A Résumé. William J. Dann and Wilburt C. Davison, Nutr. and Ped., Duke Univ., School of Med., Durham, N. C. Amer. Jour. Dis. Children, *63*, No. 2: 366–370. Feb., 1942.

The authors, in this review, show in table form the daily allowances of the various dietary essentials for children. They state that these allowances are ample for healthy children of average size and activity with normal alimentary absorption but must be increased if the child is exceptionally large or active or has fever. Also care should be taken that the vitamins are not destroyed by excessive cooking.

The authors point out that milk, eggs and meat are excellent protein sources of the ten amino acids which are indispensable in the diet.

B.E.H.

611. Prevention of Dental Caries by Massive Doses of Vitamin D Ralph Howard Brodsby, Bela Schick, and Hermann Vollmer, Sea View Hospital, New York. Amer. Jour. Dis. Children, *62*, No. 6: 1183–1187. 1941.

A group of 101 children who had tuberculosis or had been in contact with a patient with the disease were studied carefully for a period of one year to determine the effect of a single massive dose of vitamin D on the incidence of caries. They found that as the dose of vitamin D increased, the number of cavities in the teeth decreased. They divided the 101 children into 3 groups: A, control, hospital diet only; B, 305,000 U.S.P. units of vitamin D and 2,455,000 U.S.P. units of vitamin A in 30 cc. of fish liver oil concentrate in addition to hospital diet; and C, 600,000 U.S.P. units of vitamin D in form of crystalline vitamin D_2 in 1 cc. of oil in addition to the hospital diet.

They conclude, "Further studies are required on larger groups. However, the results of this study seem to indicate that the incidence of dental

caries may be markedly decreased by the administration of a single massive dose of vitamin D." B.E.H.

612. Level of Vitamin A in the Blood as an Index of Vitamin A Deficiency in Infants and in Children. J. M. LEWIS, O. BODANSKY, AND C. HAIG, Dept. Pediatrics, Beth Israel Hospital, New York. Amer. Jour. Dis. Children, *62*, No. 6: 1129–1148. 1941.

The authors conclude that, "Low levels of vitamin A in the blood in rats were found to be associated with low intakes of the vitamin and with little or no storage in the liver." They also state that 45 U.S.P. units of vitamin A per 100 cc. was the lowest found in the blood of 144 normal infants. They found that the administration of 17,000 units of vitamin A to infants during the first 6 months of life increased the vitamin A content of the blood. However, no effect on the level of vitamin A in the blood was noted when infants of 6 to 18 months old were given the vitamin A preparation for periods of 1 to 5 months. The authors are of the opinion that the level of vitamin A in the blood may be a more sensitive indicator of vitamin A deficiency than the result of the dark adaptation test. B.E.H.

HERD MANAGEMENT

613. Report of the Committee on Dairy Farm Methods. HORATIO N. PARKER, Internatl. Assn. Milk Sanit. Jour. Milk Technol., *5*, No. 3: 152. May–June, 1942.

A discussion of the fundamentals of clean milk production and dairy farm inspection is reported.

An important thing pointed out was "the unwisdom of different health departments prohibiting practices that are not objected to by others, such for instance as stipulating that covered pails should or should not be used, that milk houses should be located at certain distances from dairy barn, that surface coolers may or may not be used, etc." To avoid confusing the farmers, inspection methods should be unified. L.H.B.

614. The Progress, Results and Cost of Electric Refrigeration on Dairy Farms. L. M. GRAVES, M.D., AND REX D. BUSHONG, D.V.M., Superintendent, and Director, Bureau of Sanitary Engineering, Dept. Health, Memphis, Tenn. Jour. Milk Technol., *5*, No. 3: 131. May–June, 1942.

Mechanical refrigeration has resulted in improved quality of milk supplies. Electrically operated units were found to be more economical than gasoline-powered units. The estimated cost of electrical refrigeration varied from 6.6 cents to 12.72 cents per hundred pounds of milk.

Eighteen cities out of 28 replying to a questionnaire, reported that 50% or more of the dairies in their respective milk sheds were using electrical refrigeration for cooling. L.H.B.

615. The Use of Sodium Hypochlorite for Routine Disinfection in the Cow Shed. J. HARRISON AND A. T. R. MATTICK, Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 41–42. 1941.

When hypochlorite is added to wash water, *Streptococcus agalactiae* is killed with a reasonable margin of safety so long as the concentration of available chlorine remains above 250 p.p.m. with an exposure time of 10 seconds or over. The exposure time for the udder cloth is assured by using two cloths alternately. Determination of the rate of decline of concentration of available chlorine in wash water used for washing down the animals and in water used for dipping milking machine teat cup clusters between milkings of individual cows led to the following suggested routine.

Use two gallons of a hypochlorite solution of 800 p.p.m. available chlorine. This is enough for about 12 cows. Two buckets must be used, one containing 2 cloths for washing down the animals, the cloths being used alternately. (The one not in use is left in the disinfectant water.) The other bucket should contain 2 cloths for wiping.

After milking a cow and before starting to milk another rinse the teat cup cluster in a bucket of clean cold water, drain for 5 seconds, dip into a 2 gallon bucket of water containing hypochlorite (800 p.p.m. is sufficient for 24 teat cup clusters), plunging 3 times in 4 seconds. Drain for 3 seconds. M.W.Y.

616. The Effect of Steam, Hot Water, and Chlorine on the Life of Milking Machine Rubbers. W. A. HOY AND F. K. NEAVE, Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 39–40. 1941.

The life of liners steamed for 5 minutes was about 8 weeks, and similar results were obtained by steaming for 2 minutes. In every instance the bacteriological tests of the steamed units and the milk were satisfactory. In the case of hot water treatments, 160° F. for 10 minutes was the least destructive to the rubber but bacteriological results were not satisfactory. A temperature of 170° F. for 10 minutes gave about the same length of life as 2 minutes and 5 minutes steaming, and the results of the bacteriological tests were satisfactory. The life of liners, treated with a warm chlorine-soda solution as a combined cleaning and sterilizing process was 8½ weeks. Liners that were washed but not sterilized lasted 10 weeks. The bacteriological tests of the units treated with chlorine were occasionally unsatisfactory, but the tests of the washed units were always unsatisfactory.

It is concluded that effective sterilization by steam or hot water does not seriously reduce the life of rubber teat cup liners, and that steam sterilization is no more destructive than an efficient hot water treatment. Five minutes jet steaming is effective and allows a sufficient margin of safety in practice. M.W.Y.

617. Dairy Goat Management. G. VAN DER NOOT. N. J. Agr. Expt. Sta. Cir. 418. May, 1942.

A popular bulletin describing the four breeds of dairy goats of importance in the United States and giving specific directions for feeding and management. J.G.A.

ICE CREAM

618. The Effect of Cultures and the Relation of Acid Standardization to Several of the Physical and Chemical Properties of Ice Cream. W. H. E. REID AND L. E. SMITH. Mo. Agr. Expt. Sta. Res. Bul. 339. Jan., 1942.

The use of cultures to increase the acidity of mixes after partial neutralization was studied. Mixes were reduced in acidity from as much as 0.24 to as low as 0.06% in certain instances. Many photomicrographs of ice crystals are shown as well as many photographs of melting studies.

It was found that "cultured" ice cream had a desirable flavor and good keeping qualities. It is recommended that a starter mix be prepared by incubating at 74° F. until the acidity reached 0.85% acid, after which sufficient quantities could be added to the regular mix before pasteurization.

Standardizing the mix acidity below that which is normal for the mix requires more standardizing agent than is theoretically required, due to the buffering capacity of the mix ingredients. The standardization and subsequent development of the acidity in the mix did not affect the stability, nor the crystalline structure of the ice cream appreciably. The standardizing agent (Minsol) appeared to minimize shrinkage during storage of mixes containing 13 and 15% serum solids. C.D.D.

619. Effect of Dextrose and Sucrose Sugars upon the Properties of Ice Cream. W H. E. REID AND K. R. MINERT. Mo. Agr. Expt. Sta. Res. Bul. 339. Jan., 1942.

This bulletin describes the effect of the use of dextrose and sucrose on certain properties of ice cream mix. It is accompanied by many photomicrographs showing the structure of the resulting ice cream, ice crystals. as well as many photographs of ice cream subjected to melting tests.

The replacement of sucrose with variable increments of dextrose had no significant effect on pH, acidity, viscosity or the specific gravity of the ice cream mix. The samples containing the dextrose were slightly less sweet

than the control samples, but was not particularly noticeable until a substitution of one-third of the sucrose was made.

Dextrose affected the resistance of melting and lowered the freezing point of the mixes.

When ice cream containing sucrose only was drawn from the continuous freezer at 22° F., the ice crystals were smaller than comparable samples containing dextrose. With each increment of dextrose it was found desirable to lower the drawing temperature of the ice cream. Dextrose did not affect the number of dishes of ice cream obtained per gallon. C.D.D.

620. Report of Committee on Ice Cream Sanitation. F. W. FABIAN, Internatl. Assn. Milk Sanit. Jour. Milk Technol., *5*, No. 3: 166. May–June, 1942.

Andrew J. Krog, of Plainfield, N. J., a committee member, suggests the need of regulations governing the production of certain ingredients now being used in ice cream mix, namely, butter from plastic cream, low lactose milk solids, high conversion corn syrups and corn syrup solids, stabilizers, egg products and fruits and nuts.

A temperature of 40° F. should be considered as the maximum for mix storage.

Where novelty products are packaged in envelopes a mechanical arrangement should be required for opening the envelopes.

Wiping cloths may be sterilized in a 1–5000 solution of "Roccol" (alkyldimethyl-benzyl-ammonium-chloride). This product will not impart off flavors or odors to the product.

More adequate control of retail outlets should be practiced.

W. C. Cameron, Ottawa, Canada, reported on the ice cream situation in Canada—1941, as follows: Sales of ice cream showed a substantial increase over 1940; final statistics will probably show 1941 to be the largest on record from the standpoint of gallonage manufactured and sold. Preliminary test used for fat in ice cream is to weigh 9-gm. sample of melted ice cream into 20% ice cream test bottle, add 13 ml. of glacial acetic acid and 9 ml. of commercial sulphuric acid. If sodium alginate has been used as a stabilizer in the manufacture of the ice cream; increasing the amount of glacial acetic acid to 17 ml. will give a clearer reading. "Counter freezers do not appear to be gaining in popularity."

Ralph E. Irwin, Harrisburg, Pa., reported on developments in Pennsylvania concerning frozen desserts sanitation.

"Farms and plants contributing to preparation of ice cream are to meet the same sanitary standards as farms and plants for the preparation of milk."

A freezer may not be located in the sales room, restaurant, etc.

A small ice cream plant that may be built in sections has been prepared

for use in the manufacture and sale of ice cream at county fairs. These plants consist of a wash room, freezing room and sales room. Facilities for heating water to be used in cleansing and sterilizing equipment are provided.

Trucks built in three parts with facilities listed above, are also available for use at county fairs. L.H.B.

621. Suggestions for Cleaning Equipment in the Plant. C. W. COUGHLAN, Natl. Electric Mfrs. Co., New York. Ice Cream Rev., *25*, No. 6: 74. Jan., 1942.

The least objectionable way of removing milk stone, which is principally casein and milk salts rendered insoluble by heat, is to use a weak organic acid, such as tartaric acid, with mechanical scrubbing friction. For cleaning coil vats use one-half pound of tartaric acid crystals to 12 gallons of water. This makes a one-half per cent tartaric acid solution.

Corn sugar is also a solvent of milk stone and can be used for its removal. J.H.E.

622. Vanilla—Preparation and Types. E. G. WEED, Foote and Jenks, Jackson, Mich. Ice Cream Rev., *25*, No. 7: 26. Feb., 1942.

The author describes the culture, harvesting and curing of vanilla beans, with special emphasis on Mexican vanilla, having first-hand information on this variety as a result of trips to the Vera Cruz region.

In Mexico the picking of the vanilla pods begins in November and carries through until February. At picking time the pods contain no vanilla flavor and no vanillin. The first picking of beans comprises the wind-falls, the immature, crooked and damaged pods and often equals 15 to 25% of the entire crop. These are known as cuts or Picadura grade and lack fine flavoring properties. Next comes the ordinary, composed of short, immature pods, woody in texture, and a light reddish-brown shriveled bean. Later comes the Medium Crop of fairly good flavoring quality, followed by the Buena, or good grade. These are thoroughly seasoned pods, long, plump and dark brown in color. The last crop comprise the Prime beans. These are first quality, being dark chocolate brown, almost black in color. They are long, broad and supple and carry a rich mellow bouquet.

The Indians bring the beans to the curing stations where they are sorted as to size and quality, after which they are packed in large containers, or sweat boxes, holding from 2000 to 4000 pounds. They are allowed to sweat and ferment, with alternate sweating and drying. This is the crucial stage in the preparation of the vanilla bean. Among other little-known changes, enzymic action changes coniferyl alcohol to vanillin. About five pounds of green beans are required to produce one pound of finished cured beans.

A cured vanilla bean contains fiber, water, resin, soluble and insoluble

gums, fat, fixed oils, volatile oils, vanillin and other compounds. In extracting the flavor locked up in the beans the avoidance of heat is to be desired. The beans are chopped and packed in percolators where an alcoholic menstruum is pumped over the beans until the flavor is completely exhausted. J.H.E.

623. Sugars in Ice Cream. C. D. DAHLE, Pa. State Col. Ice Cream Rev., *25*, No. 9: 24. Apr., 1942.

Various corn sweeteners and honey can successfully be used to replace a part of the sucrose in ice cream mixes. The corn sweeteners are of different compositions and the effect on the freezing point and relative sweetness varies.

Regular corn syrup in ice cream has a sweetness of 30–40% of sucrose and the freezing point of a 15% solution is 30.55° F. Corn syrup solids are now available and the sweetness is about 50% that of sucrose. Enzyme converted corn syrup has a sweetness approximately 66% of sucrose. A 15% solution has a freezing point of 29.98° F. Corn sugar is 80% as sweet as sucrose and a 15% solution has a freezing point of 28.63° F. A number of formulas are given in which the various corn sweeteners, invert syrup and honey replace a part of the sucrose. J.H.E.

624. More Gallons Per Cabinet Hole. C. W. ESMOND, Gundlach Co., Cincinnati, O. Ice Cream Rev., *25*, No. 8: 29. Mar., 1942.

A successful sales promotion campaign of one ice cream company is described. Education must always start with the company employees. Education of dispensers is stressed also. Dealers and clerks are treated liberally with any new "feature flavor." The company also supplies hostesses to dealers to pass out samples of the feature flavor ice cream. When rainy days and slack season slow up sales, the company redoubles its efforts to help dealers by circularizing of neighborhoods with handbills. J.H.E.

625. Check upon Waste in Refrigeration. C. T. BAKER, Atlanta, Ga. Ice Cream Rev., *25*, No. 8: 28. Mar., 1942.

The danger and inefficiency of pumping ammonia liquid in a compressor is considerable. Compressor output is reduced and there is danger of damage to valves and pistons, particularly in high speed machines. Excessive piston ring wear results because of difficulty of maintaining adequate oil film on piston due to washing effect of the liquid. The appearance of liquid in the compressor discharge results in a decided drop in temperature of the discharge gas, which in turn causes a sudden contraction of flanged joints with a leaky joint likely resulting.

There are many causes for pumping liquid. The best way to detect it is

to place a reliable thermometer at the suction intake of the compressor. If such a thermometer fails to indicate a few degrees of suction superheat, but indicates saturation temperature, one may be certain that liquid is present. J.H.E.

626. Use of Sweetening Agents in the Manufacture of Ice Cream. K. M. RENNER, Tex. Tech. Col., Lubbock, Tex. Ice Cream Rev., *25*, No. 9: 44. April, 1942.

When the experimental evidence of the past few years on the use of corn sweeteners in ice cream is appraised, it is found that from 25 to 30% of the sucrose in the mix can be replaced to a definite advantage. While there is some variation between investigators regarding actual sweetening values of the various corn sweeteners, the actual variation is well within the limits of normal range.

The author is of the opinion that when the percentage replacement is from 25 to 30% of the sucrose, the pounds of other sweetener required to secure the same sweetening as one pound of sucrose is as follows: Dextrose 1.1, enzyme converted corn syrup (sweetose) 1.5, corn syrup solids 2, invert sugar solution 1, honey 1.28. J.H.E.

627. Sweetening Agents Suitable for Ice Cream. J. H. ERB, Ohio State Univ., Columbus. Ice Cream Rev., *25*, No. 9. Apr., 1942.

In replacing sucrose with various other sweeteners, the first problem encountered is that of comparative sweetness. The published results indicate no "blanket" or general value can be assigned to any sweetener. The relative sweetness appears to depend upon the concentration being compared and also the supplementary effect noted when two or more sugars are present in the same solution.

Formulas are given for replacing one-third of the sucrose with only sufficient corn sweetener to arrive at palatability and sufficient sweetness. In the case of standard corn syrup, it was found that 1.5 pounds would satisfactorily replace 1.0 pound of sucrose, when one-third of a 15% sugar mix was replaced with corn syrup. J.H.E.

628. Refrigeration in Ice Cream Factories and Other Dairy Plants. L. C. THOMSEN, Univ. Wis., Madison. Ice Cream Rev., *25*, No. 6: 18. Jan., 1942.

Dairy plants have a choice of five major systems of refrigeration: (1) the direct expansion system, (2) the flooded type, (3) brine system, (4) the "sweet water" system and (5) the "sweet water" ice storage system. Each has certain advantages and disadvantages, which are discussed. The sweet water system has had much popularity in recent milk plant installations.

Calculations and charts are given proving that for greatest capacity and lowest operating costs the evaporating side of the system should be run with

the maximum suction pressure possible, still obtaining the desired temperatures. A room may, under certain conditions, actually be cooled to a lower temperature more rapidly by increasing the temperature of the refrigerant in the evaporating coils, than by calling for more refrigeration by turning down the thermostat or by operating at a lower suction pressure. In addition, the cooling operation will cost less. This is because at higher suction pressures the compressor will handle a greater weight of gas with each displacement of the cylinder.

It is recommended that greater use be made of gauges and instruments in refrigeration to check on proper operation. J.H.E.

MILK

629. New Tests on Refrigerated Milk. JOHN E. NICHOLAS AND T. G. ANDERSON, Pa. State Col., State Col. Pa. Refrig. Engin., 44, No. 1: 23. July, 1942.

Bacteriological analyses in different strata in 10-gallon milk cans is a function of temperature. A 10-gallon milk can was fitted with sampling tubes located at three levels, top of breast, at middle of body, and near the bottom of the body. At each level three tubes were inserted to three depths, one at the geometric vertical center, one near the wall of the can, and one reaching to vertically midway between. This arrangement rendered it possible to remove samples of milk for temperature and bacteria determinations at the several levels. The bacteriological analyses reported covered three different sets of conditions under which the milk was cooled; namely, (a) with a large amount of initially available refrigeration and the water both in motion, (b) with a large amount of initially available refrigeration, but no motion of the water bath, (c) with both the milk can and water bath in motion simultaneously.

The most satisfactory results were obtained when a water to milk ratio of 6 to 1 was employed with a heavy ice bank and the water bath agitated, the temperature in the top layer of milk having been lowered from 90° to 46° F., while that of the bottom layer had reached 37° F. in one hour. During this time, the water bath had remained below 35° F. and continued to do so throughout the succeeding cooling hours. The average bacterial content in most instances decreased, the better results being obtained under the first condition of cooling.

The motion of can in addition to water-bath agitation would not seem to give any material advantage though a lower ratio of water to milk was employed and not as much ice bank.

Milk cooled under the condition of heavy ice bank, without bath agitation, but with a water bath to milk ratio of 6 to 1, while only reaching 50° F. in the top layer in 4½ hours, revealed very little change in average bacteria

content of the milk after 15 hours in the cooler as compared to that of the fresh milk.

The results indicate that it is particularly important to reduce the temperature of the top layer of the milk to below 50° F. as rapidly as possible because it was found that there was a variation in the ratio between the organisms in the fresh milk and the cream layer from as low as 5.5 to 1 to as high as 173 to 1.

When homogenized milk was studied, it was found that the bacterial content remained practically the same under conditions in these experiments. L.M.D.

630. Thermoduric Bacteria in Milk and a Simple Remedy Against Them. M. J. PRUCHA, Dept. Dairy Husb., Univ. Ill. Milk Plant Monthly, *31*, No. 4: 38. April, 1942.

A large number of different species of bacteria belong to the thermoduric group, a group able to withstand in large part ordinary pasteurization exposures. While the general belief is that the problem of thermoduric bacteria is primarily on the farm, not all milk plants are innocent. Milk stone is invariably a source of contamination. The remedy to eliminate or reduce the number of thermoduric bacteria consists in (1) having good, smooth-surface equipment, (2) washing utensils and equipment properly so no bacterial food remains, (3) giving the utensils satisfactory bactericidal treatment, and (4) avoiding prolonged heating of milk at temperatures between 100° and 143° F. G.M.T.

631. The Problem of Controlling Rancidity in Milk. N. P. TARASSUK, Dairy Inds. Div., Univ. Calif., Davis, Calif. Milk Plant Monthly *31*, No. 4: 24. April, 1942.

Rancidity in milk may result from spontaneous or induced activity of lipase present in milk. Apparently two lipases exist in milk, the one present in late lactation milk which acts spontaneously and the other present in all milk which is activated by (1) prolonged and violent agitation of warm milk, (2) rewarming precooled milk to 86° F. and recooling to 50° F., and (3) homogenization. By warming the milk to 105° F. development of rancidity is prevented although the enzyme is not destroyed. The feeding of green feed to cows in late lactation remedies the difficulty with spontaneous development of rancidity. By mixing normal milk with "lipase milk" in the proportion of 2 to 1 before any rancidity develops prevents the development of rancidity. G.M.T.

632. Preparation and Merits of Churned Buttermilk. C. L. ROADHOUSE, Dairy Dept., Univ. Calif., Davis, Calif. Milk Plant Monthly, *31*, No. 7: 32. July, 1942.

A method for making a pleasing-flavored, churned, cultured buttermilk, containing butter granules, between 1 and 2 per cent of milk fat, and which does not "whey off" readily has been developed. The method consists basically in (1) pasteurizing the skim milk between 185° and 190° F. for 1 hour, (2) pasteurizing the cream separately at 145° F. for 30 minutes, (3) adding 0.75% fat in the form of cream along with approximately 1% starter, setting the milk at 70° F. and ripening to an acidity of 0.70 to 0.80% before churning, (4) churning at 68° to 72° F. by circulating the culture through a centrifugal pump back to the vat, (5) salting at the rate of 2.2 ounces salt to each 100 pounds of milk, (6) adding 0.025% sweet cream to the granuled product, and finally, (7) cooling the buttermilk to 40° F. or below. Butter color is added to give the granules a high color. Butter granules remain more evenly distributed if the buttermilk is not bottled until after it is stored several hours. G.M.T.

633. Homogenized Milk. G. M. TROUT, Mich. Agr. Expt. Sta., East Lansing, Mich. Milk Dealer, *31*, No. 10: 30–31, 51–53. July, 1942.

The problems incident to the production and use of homogenized milk are discussed under behavior changes and processing. The problems under behavior changes are classified as follows: 1. Processing. 2. Packaging. 3. Distribution. 4. Laboratory Control. 5. Cooking. 6. Utilization of Returns. The processing problems are: 1. Rancidity. 2. Sedimentation. 3. Increased Bacterial Counts. 4. Inefficient Homogenization-meeting Standards. 5. Cream Line. The following conclusion is made: While homogenized milk apparently gives rise to numerous problems, these problems fortunately may be solved. Let it be recognized that problems exist in the processing and distribution of any product and homogenized milk is no exception. The general acceptance of homogenized milk indicates that the problems are not insurmountable. C.J.B.

634. The Use of Laboratory Pasteurization in Solving Milk Problems. ELIAS B. BOYCE, HERMAN C. LYTHGOE, ELLA K. RUGGLES AND ROBERT LANE, Div. Food and Drugs, Dept. Health, Boston, Mass. Jour. Milk Technol., *5*, No. 3: 146. May–June, 1942.

Laboratory pasteurization with standard agar plate counts on the milk before and after pasteurization when compared with the plate count of the plant pasteurized sample will give a good indication as to the efficiency of the pasteurization process carried on in the plant.

In many cases of high bacterial count on pasteurized milk the cause has been found to be due to the presence of thermoduric organisms in the raw supply. Milking machines have been found frequently to be the cause of these organisms in the raw milk. Laboratory pasteurization was also shown

to give a "clean bill of health" in some cases to the producer. Showing conclusively that the trouble was in the plant. L.H.B.

635. Chemical Taste in Milk. CHAS. PALEY, Certified Labs., Inc., New York, N. Y. Jour. Milk Technol., *5*, No. 3: 165. May–June, 1942.

Reporting an incident where the use of a chlorine rinse for dairy equipment caused a chemical flavor to be imparted to the first milk over the equipment. L.H.B.

636. Relation of Ascorbic Acid and of Oxygen to Oxidized Flavor in Milk. J. C. LEEDER AND E. O. HERREID, Vt. Agr. Expt. Sta., Burlington. Vt. Agr. Expt. Sta. Bul. 481. 1942.

The milk used in this study was from the university herd and averaged 3.85 and 3.91% fat during the two trials. Pasteurization was carried out in a 30- and a 1-gallon stainless steel vat. Cooling was done either in the pasteurizer or over tinned copper surface cooler.

Oxygen and ascorbic acid determinations and flavor observations were made on the pasteurized milk held for definite storage periods. The surface-cooled milks lost more ascorbic acid and developed more oxidized flavor than did those cooled in the pasteurizing vat.

The ascorbic acid content of the mixed herd milk was not altered by changes from winter to summer feeding but the oxidized flavor occurred more often during the barn feeding. The off flavor appeared when approximately $\frac{2}{3}$ of the ascorbic acid was destroyed in 15 cases.

Ascorbic acid is probably not the only constituent related to the development of oxidized flavor. Since the theoretical ratio of oxygen/ascorbic acid disappearance is disturbed it is evident that other oxidizable constituents are involved. P.H.T.

637. The Bacterial Flora of Milk Pasteurized by the "In Bottle" Process. A. ROWLANDS, Midland Agr. Col., AND A. L. PROVAN, Harper Adams Agr. Col. Proc. Soc. Agr. Bacteriologists (Eng.), 29–30. 1941.

Forty-two pint samples of milk were examined during storage at 15.5° C. The mean keeping quality was 2.82 days. Acid-producing organisms were predominant in fresh samples, but at the end of 48 hours were superseded by alkali-producing and peptonizing types, which formed only a relatively small proportion of the total flora of fresh samples. After 72 hours, organisms producing an acid clot reaction in litmus milk predominated. M.W.Y.

638. Chlorine Sterilization in Practice and in the Laboratory. F. K. NEAVE AND W. A. HOY. Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 37–38. 1941.

Tinned trays were artificially infected with a 10% milk suspension of *Staphylococcus aureus* and dried at 37° C. for 1 hour before use. Chlorine solutions were poured on to the trays, which were mechanically agitated. The time taken for a reduction of 99.9% in the number of bacteria on trays rinsed with solutions containing 400, 200, 100 and 25 p.p.m. available chlorine was respectively 1, 1.3, 2.5, and 6.5 minutes. All tests were made with solutions prepared in tap water at a temperature of 55 to 60° F.

M.W.Y.

639. The Methylene Blue and Resazurin Tests for Pasteurized Milk. A. ROWLANDS, Midland Agr. Col., AND A. L. PROVAN, Harper Adams Agr. Col. Proc. Soc. Agr. Bacteriologists (Eng.), 36. 1941.

The results from 160 samples showed that neither the methylene blue nor the resazurin test at 37° C. is a reliable measure either of recontamination or of the keeping quality of pasteurized milk when the samples are examined immediately after removal from cold storage on the morning after processing. Results of the methylene blue test at 15.5° C. indicate that some preliminary incubation is necessary before either of these tests is applied. M.W.Y.

640. Bacteriological Indices of the Sanitary Quality of Market Cream. ELIZABETH D. ROBINTON, EARLE K. BORMAN, AND FRIEND LEE MICKLE, Bur. of Lab., Conn. State Dept. Health, Hartford, Conn. Amer. Jour. Pub. Health, *32*, No. 5: 464–470. 1942.

A total of 730 samples of raw, import and retail pasteurized cream were tested for the enumeration of bacteria by the following laboratory methods: 48 hours plate count at 37° C., at 55° C., and at 20° C., 4 days plate count at 8° C., and the direct microscopic clump count. The results indicate that the direct microscopic clump count is the most satisfactory and practical method for this purpose, especially if the sample, when pasteurized, is supplemented by a coliform determination and a phosphotase test. The direct microscopic method is the best method for detecting spore-forming organisms which ordinarily do not produce colonies on agar plates incubated at 8° C., 20° C., 37° C., and only rarely develop at 55° C. M.W.Y.

641. San Francisco's Pasteurization Ordinance Finally Upheld. EDITORIAL. Amer. Jour. Pub. Health, *32*, No. 5: 470. 1942.

An ordinance banning the sale of milk in San Francisco, except certified, unless it was pasteurized, which was passed in 1933 by the Board of Supervisors, was finally upheld as a proper exercise of the police power by a decision of the Supreme Court of California rendered April 2, 1942.

M.W.Y.

642. Milk Delivery Costs. ANONYMOUS. Milk Dealer, *31*, No. 8: 54. May, 1942.

A study made by the California Department of Agriculture showed that: When more than case lots are delivered at each wholesale stop, time studies revealed, the average delivery cost is 0.8383 of a cent per quart and in those instances where less than case lots are delivered the average wholesale delivery cost is 1.4604 cents, or a saving of 0.622 of a cent per quart. Study of costs pertaining to savings in multiple retail delivery sales reveal cost per single quart is 3.5758 cents, while the cost per quart in multiple sales is 2.7715 cents, or a saving of 0.8043 of a cent per quart.

Tables are presented showing "breakdown" information on wholesale and retail delivery costs. C.J.B.

643. "Vemp" Campaign. ANONYMOUS. Milk Dealer, *31*, No. 8: 33, 70. May, 1942.

A survey conducted by Milk Foundation, Inc., of Chicago, in which 1,552 housewives in Chicago, Cleveland, Indianapolis, Columbus, and Dayton revealed that 43% of the adults interviewed did not drink milk because they disliked it; 44% of the adult males and 49% of the adult females drank no milk at all; 9% of children 6 to 12 years of age and 13% of children 13 to 18 years of age drank no milk at all.

As a result of this survey a promotion campaign to sell more milk has been started. The campaign is built around "Vemp," a word made up of the first letters of the words "Vitamins," "Energy," "Minerals," and "Proteins."

The following steps for action are suggested: 1. Stress importance of adequate daily amounts of milk to tie in with present government campaign for adequate nutrition. 2. Direct campaign to adults because adults account for two-thirds of population and for 76% of milk deficiency below recommended minimums. 3. Direct campaign to both men and women because consumption deficiency is split nearly equally. 4. Feature milk drinking but keep it above beverage class by stressing nutritional superiority to meet beverage competition. 5. Play up food values. 6. Play up between-meal and bedtime drinking because this market is only partially developed and competition is weaker here than at meal time. 7. Meet the taste factor squarely, since taste loomed large in the survey as a reason for not drinking milk. 8. Play up the economy of milk. 9. Stress refreshing quality of milk in hot weather. 10. Tie into general nutrition-consciousness and government program. C.J.B.

644. Studies Relating to Soft Curd Milk. J. C. MARQUARDT, N. Y. Agr. Expt. Sta., Geneva, N. Y. Milk Dealer, *31*, No. 8: 27–28, 72. May, 1942.

A procedure for predicting soft curd values is described. Perfections have been made in gravitation studies involving 3-layer components based upon the creaming factor for normal milk. Composition and curd character in components of normal and homogenized milk and heat treatments to produce soft curd milk have been studied. The following conclusions are drawn: 1. A curd value in Hill units of 20 or less can be predicted when a value of 10 Hill units or less is attained after a two-minute setting period at 90° F. 2. Three-layer component gravitations have advantages over two-layer separations in measuring homogenization efficiency. 3. The fat, solids and ash are evenly distributed in various components of gravitated homogenized milk. This is not true with unhomogenized milk. 4. The curd strength of gravitated homogenized milk components is very uniform. This is not true with unhomogenized milk. 5. It is possible by employing certain heat treatments to produce milk that has a curd strength comparable to that of properly homogenized milk. 6. Homogenization decreases the normal setting time of milk. C.J.B.

PHYSIOLOGY

645. Factor of Age in the Rate of Absorption of, and in Mammary Stimulation by, Testosterone Monopropionate Pellets in Rats. THOMAS R. FORBES, John Hopkins Univ., Endocrinology, *30*, No. 5: 765. May, 1942.

Testosterone monopropionate pellets weighing 8.4 mg. to 10.5 mg. were implanted in 4 male and 5 female litter-mate rats aged 16 days; in 4 males and 5 females, aged 47 and 54 days; and in 5 males and 4 females, aged 12 months. Each rat received one pellet and gross observations were made of the mammary glands 12 days after implantation. Neither males nor females of the 16-day-old group showed any gross mammary development. Three males and 2 females of the 47- and 54-day-old group showed no appreciable mammary stimulation. The mammary glands of one male and 3 females showed slight stimulation. The mammary glands of all 5 males and 2 females of the 12-month-old group were moderately developed and the other 2 females showed marked mammary growth. R.P.R.

646. Effects of Estrogens upon the Young of Injected Lactating Rats. CHARLES K. WEICHERT AND SYLVIA KERRIGAN, Univ. Cincinnati. Endocrinology, *30*, No. 5: 742. May, 1942.

The litters of each of 15 rats were reduced to 3 males and 3 females and the mother rats injected with an estrogen immediately after parturition. The weights of each mother and litter were recorded daily. The young grew normally for 5 or 6 days and then failed to gain weight at the normal rate.

On the 14th day the weights of the young of injected mothers ranged from 11 to 17 gm. as compared with an average weight of 26.5 gm. for those of untreated controls. Lactation did not seem to be suppressed inasmuch as milk exuded from the cut surface of the mammary glands at autopsy and sections showed alveoli distended with secretion. Milk was also present in the stomachs of most of the young when they were autopsied. It was thought that loss of maternal instinct probably accounted in part for failure of the young to grow normally. R.P.R.

647. Studies Concerning the Mechanism Controlling the Initiation of Lactation at Parturition. I. Can Estrogen Suppress the Lactogenic Hormone of the Pituitary? JOSEPH MEITES AND C. W. TURNER, Univ. Mo. Endocrinology, *30*, No. 5: 711. May, 1942.

The daily administration of 2 mg. of either diethylstilbestrol or testosterone propionate to lactating rats for the first 6 days postpartum increased the pituitary lactogen content, caused some reduction in the amount of milk present in the mammary glands, and resulted in the death of 38 and 11% respectively of the young rats. Pituitaries from lactating rats which were not suckled for the first week after parturition contained 50% less lactogen than suckled rats and the mammary glands from these rats were practically devoid of milk. The injection of 0.0125 to 100 mg. of diethylstilbestrol over a 5-day period into normal immature and mature male guinea pigs increased the lactogen content of their pituitary glands up to 438%, the smaller dosages being more effective than the larger dosages. R.P.R.

648. Studies Concerning the Mechanism Controlling the Initiation of Lactation at Parturition. II. Why Lactation is not Initiated During Pregnancy. JOSEPH MEITES AND C. W. TURNER, Univ. Mo. Endocrinology, *30*, No. 5: 719. May, 1942.

Immature female guinea pigs weighing approximately 300 gm. were injected with either one mg. of progesterone, 25 to 200 International Units of estrone, or one mg. of progesterone plus 25 to 200 I.U. of estrone daily for 5 days. Progesterone alone had no effect on the lactogen content of the pituitary, estrone alone caused definite increases in pituitary lactogen content, and certain combinations of progesterone and estrone either entirely prevented or reduced the increase in pituitary lactogen content which was obtained with estrone alone. It was suggested that during pregnancy the progesterone-estrogen ratio is such that progesterone overrides the lactogen-stimulating effects of estrogen and that this action accounts for the fact that the pituitary lactogen content remains as low during pregnancy as in the nonpregnant state. R.P.R.

649. Studies Concerning the Mechanism Controlling the Initiation of Lactation at Parturition. III. Can Estrogen Account for the Precipitous Increase in the Lactogen Content of the Pituitary Following Parturition? JOSEPH MEITES AND C. W. TURNER, Univ. Mo. Endocrinology, *30*, No. 5: 726. May, 1942.

The effect of the length of estrogen treatment on pituitary lactogen content was determined in 40 male guinea pigs. Eleven similar pigs receiving no treatment served as controls. All experimental pigs received the same dosage of estrone (3000 I.U.) over periods of time varying from one to 30 days. A 142 and 204% increase in pituitary lactogen content was obtained in the groups injected for 1 and 2 days respectively and a 371% increase was secured in animals injected for 5 days. The animals injected for 10 to 30 days did not show a significantly greater increase over those injected for 5 days. Four New Zealand White rabbits were hysterectomized on the 20th day of pregnancy and they were killed 5 days later. Individual assays of the pituitaries showed that an average increase of 83% in pituitary lactogen content had been obtained. R.P.R.

650. Progesterone-Like Activity of Some Steroid Compounds and of Diethylstilbestrol in Stimulating Mammary Lobule-Alveolar Growth. JOHN P. MIXNER AND CHARLES W. TURNER, Univ. Mo. Endocrinology, *30*, No. 5: 706. May, 1942.

Various compounds were assayed for their mammary lobule-alveolar growth promoting properties by injecting them simultaneously with a standard dosage of estrone into ovariectomized virgin mice. The criterion of response was the percentage of mice showing a minimum amount of lobule-alveolar growth after 10 daily subcutaneous injections. Employing progesterone as a standard, pregneninolone had an activity of approximately one-half, desoxycorticosterone acetate and dehydroandrosterone of one-third, diethylstilbestrol of one-fourth, acetoxy-pregnenolone of one-sixteenth, and methyl testosterone of one twenty-fifth, that of progesterone. Testosterone and testosterone propionate in dosages up to 10 mg. failed to stimulate lobule-alveolar growth. R.P.R.

651. Effect of the Gonadotropic Substance of Pregnant Mare's Serum on the Blood Plasma-Ascorbic Acid of the Castrate Bovine. FREDERICK N. ANDREWS AND RALPH E. ERB, Purdue Univ. Endocrinology, *30*, No. 5: 671. May, 1942.

The injection of 1000 to 2000 rat units of pregnant mare's serum into grade Hereford steers approximately two years of age was followed by decreases in venous blood plasma-ascorbic acid of 42 and 56% respectively within a period of 5 hours. The recovery of blood-plasma-ascorbic acid to approximately pre-injection levels required 44 to 68 hours. R.P.R.

652. Progesterone Effect on Pituitary Lactogen Content and on Mammary Glands of Ovariectomized Rats. R. P. Reece and J. A. Bivins, N. J. Agr. Expt. Sta. Soc. Expt. Biol. and Med. Proc., *49*, No. 4: 582. Apr., 1942.

The daily injection of 15 mg. of progesterone for 10 days into sexually mature spayed rats increased the lactogen content of their pituitary glands, caused no significant increase in pituitary weight, and induced moderate development of the lobule-alveolar system and limited secretory activity of the mammary glands. The daily administration of 15 mg. of progesterone plus 33 micrograms of estrogen for 10 days caused extensive lobule-alveolar growth of the mammary glands. The secretory activity of the glands was somewhat greater than that following progesterone treatment. This treatment was less effective than estrogen but more effective than progesterone in increasing the lactogen content of the pituitary gland. R.P.R.

653. Lobule-Alveolar Growth of Mammary Glands of Hypophysectomized Female Rats. Ralph P. Reece and Samuel L. Leonard, N. J. Agr. Expt. Sta. and Cornell Univ. Soc. Expt. Biol. and Med. Proc., *49*, No. 4: 660. Apr., 1942.

Twenty sexually mature virgin rats were ovariectomized and hypophysectomized. Beginning on the day following operation 6 of the rats were injected subcutaneously daily for 15 days with 300 micrograms of testosterone propionate and the remaining 14 rats received subcutaneously 300 micrograms of testosterone propionate plus 0.5 cc. of growth hormone intraperitoneally daily for 15 days. The rats were sacrificed on the day after the last injection and the right abdominal mammary glands studied as whole mounts. The mammary glands of the testosterone-treated rats showed involutionary changes but the lobule-alveolar system of the mammary glands was developed in 12 out of 14 rats receiving the combined treatment. R.P.R.

654. Assay of Adrenals for Lactogenic Hormone. V. Hurst, J. Meites and C. W. Turner, Univ. Mo. Soc. Expt. Biol. and Med. Proc., *49*, No. 4: 592. Apr., 1942.

Two commercial adrenal cortical extracts, whole untreated rabbit adrenals, and isoelectric precipitates of beef, hog and rabbit adrenals were assayed for lactogenic hormone by the intradermal pigeon crop gland method. None of the adrenal compounds was found to contain the lactogenic hormone. R.P.R.

655. Effects of Testosterone Propionate in Spayed Female Rats. G. L. Laqueur, Stanford Univ. Soc. Expt. Biol. and Med. Proc., *49*, No. 3: 425. Mar., 1942.

Six rats were spayed during estrus and 6 rats were spayed on the second day of diestrus. 4 mg. of testosterone propionate were administered subcutaneously every other day over a period of 20 days. The animals were killed 24 hours after the last injection. The mammary glands showed an increase of interlobular fibrous tissue, overgrowth of the ducts, some evidence of secretion but no proliferation of the alveoli. R.P.R.

656. Effect of Certain Hormones and Drugs on the Perfused Mammary Gland. W. E. PETERSEN, University of Minnesota. Soc. Exp. Biol. and Med. Proc., *50*, No. 2: 298. June, 1942.

The experiments were carried out on bovine mammary glands perfused according to a previously reported technic. Glands were obtained from a packing plant and the teats were cannulated to permit drainage of the milk immediately after the perfusion started and the cannula left in situ during the experiment. Blood pressure was maintained at 110 mg. Hg pressure and the tested substances were injected into the arterial blood as it entered the gland. Pitocin injections were made following the injection of other substances as a test for the completeness of the milk ejection. Pitocin and acetylcholine caused complete ejection of milk, the former caused a decrease in blood flow while the latter had no influence on blood flow. Pitressin caused an incomplete ejection of milk and a marked decrease in blood flow. Epinephrin and histamine caused a partial ejection of milk and a marked decrease in blood flow. Ergonovine had no effect on milk ejection but caused some vasoconstriction. Acetyl-B-methylcholine caused a partial ejection of milk and carbamylcholine had no effect on milk ejection. Atropine slightly increased the blood flow and completely prevented any response to acetylcholine and acetyl-B-methylcholine but had no influence on the action of pitocin or pitressin. The results were believed to indicate that the mammary gland is innervated by the parasympathetics as well as the sympathetics. R.P.R.

657. Forced Ovulation of Normal Ovarian Follicles in the Domestic Fowl. R. M. FRAPS, M. W. OLSEN AND B. H. NEHER, U.S.D.A. Soc. Exp. Biol and Med. Proc., *50*, No. 2: 308. June, 1942.

Ovulation of the normally growing follicle of the hen's ovary was induced prematurely by as much as 17 hours by the intravenous injection of several hormones. A luteinizing principle from horse anterior pituitaries caused ovulation of follicles 10 to 11 hours prior to time of normally expected ovulation in 100% of injected hens. Prehysin, Gonadin, and Anteron were effective when injected at sufficiently high levels. At certain submaximal injection levels the percentage of ovulating hens decreased with increasing prematurity of the ovarian follicles and this effect was more pronounced in

follicles of relatively great prematurity at the time of effect of the injected hormone. R.P.R.

658. Time Required for Induction of Ovulation Following Intravenous Injection of Hormone Preparations in Fowl. R. M. FRAPS, G. G. M. RILEY AND M. W. OLSEN, U.S.D.A. Soc. Exp. Biol. and Med. Proc., *50*, No. 2: 313. June, 1942.

Ovulation time following intravenous injection of gonadotropic hormones into pretreated and normally laying hens was determined. The interval in pretreated hens averaging more than 2 ovulations per hen at time of autopsy was 6.8 hours. Ninety per cent of 69 normally laying hens ovulated from 6.5 to 8.5 hours following injection and the remaining 10% required up to 9.6 hours. The minimal ovulating interval for pretreated hens was 6.1 hours and for non-pretreated hens, 6.5 hours. R.P.R.

659. Functions of the Adrenal Cortex. FRANK A. HARTMEN, Ohio State University. Endocrinology, *30*, No. 6: 861. June, 1942.

Cortilactin, an extract of the adrenal cortex, was recently prepared by isoelectric precipitation. This factor enables lactating rats kept in good condition by other adrenal fractions to lactate adequately so that all of their pups grow at a normal rate. Cortilactin does not affect glyconeogenesis and full replacement has been obtained by means of Kendall's compound E. The author suggests that it seems quite possible that more than one factor may be effective in lactation. R.P.R.

660. Mammogen and Unilateral Mammary Gland Growth in the Rabbit. A. A. LEWIS AND C. W. TURNER, University of Missouri. Endocrinology, *30*, No. 6: 985. June, 1942.

Unilateral percutaneous treatment of male rabbits with estrone or diethylstilbestrol caused either greater mammary gland development on the treated side or development of treated glands only. In 2 of 3 rabbits the injection of estrone and the unilateral percutaneous application of turpentine caused the development of larger mammary glands on the turpentine-treated side. Turpentine-treated mammary glands from diethylstilbestrol-injected rabbits, however, did not show any increased size over that of untreated glands. One rabbit treated unilaterally percutaneously with turpentine had several mammary glands showing some duct development on the treated side but 3 additional rabbits gave negative results. Other irritants, oil of myrrh, medical turpentine, oil of cantherides, and eucalyptus oil, applied unilaterally to the skin gave negative results. R.P.R.

661. Pituitary Weight in Growing New Zealand White Rabbits in Relation to Live Weight. H. H. KIBLER, A. J. BERGMAN AND C. W.

TURNER, University of Missouri. Endocrinology, *31*, No. 1: 59. July, 1942.

Pituitary and associated body weights for growing New Zealand White rabbits were presented in a logarithmic chart. A covariance analysis of pituitary to body weight relations established a statistically significant difference due to sex. The ratio of pituitary to body weight was found to decline with increase of body weight during growth. Fitted equations relating pituitary and body weights were compared with curves for mature animals of different species for a similar range of body weights. Prediction tables of pituitary weight from body weight and for proportioning pituitary or metabolism stimulating injections according to pituitary weight were presented. R.P.R.

662. Influence of Some Steroid Hormones on Lactation in Adrenalectomized Rats. ROBERT GAUNT, W. J. EVERSOLE AND E. C. KENDALL, New York University and Mayo Foundation. Endocrinology, *31*, No. 1: 84. July, 1942.

The influence of various steroids of the adrenal cortex and of the gonads on the deficient lactation of adrenalectomized rats was studied. Indications were that all substances known to relieve adrenal insufficiency were somewhat helpful. Desoxycorticosterone acetate gave variable results as it was helpful in some but not in all rats and in no case maintained lactation equal to the average control figure. Normal lactation for the first 17 days was obtained with compound E and the whole extract of the adrenal cortex. A tentative conclusion was drawn that full mammary secretion is dependent on those adrenal factors which affect primarily carbohydrate metabolism. Estrogen and androgen inhibited lactation even in small doses but progesterone did not inhibit lactation. R.P.R.

663. Non-Effect of Hysterectomy upon the Mammary Gland of the Monkey. HAROLD SPEERT, Carnegie Institution of Washington and Johns Hopkins Hospital. Endocrinology, *31*, No. 1: 97. July, 1942.

Hysterectomy was performed on an adult rhesus monkey on day 22 of an ovulatory menstrual cycle and observed over a period of 6 months. Hysterectomy failed to delay involution of the corpus luteum and had no effect on the mammary glands. R.P.R.

664. Mammary Growth in Male Mice Fed Desiccated Thyroid. W. U. GARDNER, Yale University. Endocrinology, *31*, No. 1: 124. July, 1942.

The mammary glands of 13 out of 14 intact male mice fed desiccated thyroid mixed with their feed, 1.5 mg. per kg., showed proliferation of the

ducts and hyperplastic end-buds. Similarly treated castrated male mice showed no mammary growth. The adrenal glands of the thyroid-fed intact or castrated male mice were greatly increased in weight and the kidneys and livers were also larger than those of the controls. The amounts of desiccated thyroid consumed were insufficient to reduce the gain in body weight below that of the controls. R.P.R.

655. Effects of Lactogen on Normal and Adrenalectomized Female Rats. CHARLES E. TOBIN, University of Rochester. Endocrinology, *31*, No. 2: 197. Aug., 1942.

Daily subcutaneous injections of lactogen for a period of 4 to 10 days to normal rats caused maintenance of corpora lutea which apparently secreted progestin. Bilaterally adrenalectomized rats similarly treated from the day of operation until death or until the 15th day for those which survived longer had a significantly prolonged survival time when treated with one lactogen (Difco) but not with the other one (Schering) and showed better maintenance of follicles and corpora lutea than untreated controls.

R.P.R.

666. Relation of Thyroid to Growth. I. Effects of Crystalline Thyroxin upon Rate of Growth, Food Intake and Body Composition of Female Albino Mice. MARVIN KOGER, VICTOR HURST AND C. W. TURNER, University of Missouri. Endocrinology, *31*, No. 2: 237. Aug., 1942.

Virgin mice weighing from 13 to 16 gm. were injected subcutaneously daily with crystalline thyroxin in doses ranging from 0.015 to 0.04 mg. The thyroxin-treated animals repeatedly gained an average of 28% more weight during a 5-week period than did the controls. The maximum difference was attained in about 5 weeks after which time the difference gradually became less but the body weight of the thyroxin-treated mice did not exceed that which was finally attained by the controls. The daily food intake of the thyroxin-treated mice was approximately 25% above that of the controls. The carcasses of the injected mice had a higher percentage of water and nitrogen and a lower percentage of fat than did those of the controls. Thyroxin-injected mice stored more nitrogen and gained more in body weight per unit of food intake than did controls for the first few weeks of treatment. After the rapid gains had subsided the controls were more efficient than were treated mice. Control mice stored more fat and more energy per unit of food intake than did the thyroxin-injected animals. The practical application of the results to the livestock industry was discussed.

R.P.R.

667. Pituitary Weight of Growing Male Albino Rat Related to Body

Weight. JOHN P. MIXNER AND CHARLES W. TURNER, University of Missouri. Endocrinology, *31*, No. 2: 261. Aug., 1942.

One hundred and thirty-five male albino rats of the Wistar strain were used to obtain data relating pituitary and body weights. Data were correlated by fitting the data by the product moment method to the equation $Y = a + bX$ and by fitting the points on the line of means by the method of least squares to the equation $Y = aX^b$. The latter equation indicated that pituitary weight was not directly proportional to body weight but varied with the 0.68 power of body weight. R.P.R.

668. Reconsideration of the Significance of Some Plant Characteristics in Relation to Yield of Gonadotropic Material. JOHN W. MITCHELL, RUBIN BORASKY AND JAMES T. BRADBURY, U.S.D.A. Endocrinology, *31*, No. 2: 283. Aug., 1942.

Data collected during the last 3 years showed that ovulation was induced in rabbits as the result of the administration of some plant juice extracts during the spring and summer months as measured by the arbitrary standards previously described. Many negative results were observed during all seasons of the year. Positive assays were not found to be correlated with any of the plant characteristics noted such as age, succulency, hydrogen-ion concentration of the juice, or with any factor of the environments under which the plants were grown. R.P.R.

MISCELLANEOUS

669. The Application of Plate Type Evaporators to Truck Bodies. ALBERT F. SAWYER, Development Engineer, Dole Refrigerating Co., Chicago, Ill. Refrig. Engin., *43*, No. 6: 349. June, 1942.

Vacuum plates consist of two sheets of metal forming a flat box within which are evaporative coils of steel tubing. A vacuum is drawn on the space between the metal sheets which draws them against the tubing. Edges and corners are welded to form a hermetically sealed compartment. If holdover capacity is desired, a eutectic solution is placed in the inter-plate space before exhausting. Two types of holdover plates are made, those of comparatively thin cross section for 4- to 5-hour holdover effect, and others of $2\frac{3}{8}$-inch thickness for 12- to 18-hour holding. These latter require spacers over the coils to build up to the required thickness.

Eighteen-degree solution is recommended for temperatures of 36° F., while – 6° solution is required for temperatures of 0° to 5° F. K factor for the plates is set at 2 B.t.u., and a temperature difference of 18° for the 36° F. work, while a t.d. of 15° is selected for the low temperature duty involved in ice cream trucks or trucks handling low temperature frozen foods. This

is because of the inherent refrigeration in frozen foods and ice cream, the latter loading in at – 10° F. or lower.

Low temperature holdover plates may be located in practically any position with satisfactory results, while high temperature plates are best located on side walls with drip trough placed below them. Long distance transportation trucks must be equipped with refrigerating units powered either by separate power plants or power take-offs, and the system designed to operate about 18 hours out of 24, using either dry plates or partial holdover plates. L.M.D.

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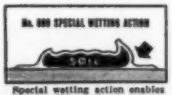

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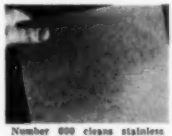
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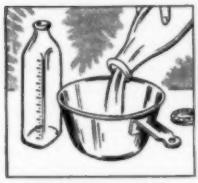
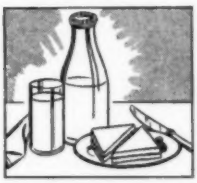
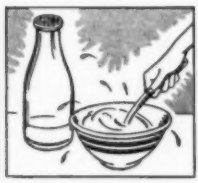


BABY'S FORMULA: Pure milk guarded against danger of contamination by Seal-Kap.

BREAKFAST: Bottle opened. Seal-Kap *re-seals*, completely covering the pouring lip.



LUNCH: Bottle opened again. Seal-Kap still on guard against dirt, dust and ice-box odors.



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FLAV-O-LAC FLAKES (shown) produce a quart of the finest starter on a single propagation. Single bottles $2.00.

SPECIAL FLAV-O-LAC FLAKES "40"
produce 40 quarts of starter on a single propagation. Single bottles $3.00

Free Culture Mannual of Fermented Milk Products on request.

Pioneers in Spectro-chemical, Chemical and Fluoro-photometric Determinations of Vitamins A, B_1, B_2, Nicotinic Acid, Pantothenic Acid, B_6, C & E in Dairy and Food Products. (Vitamin D excluded) inquiries invited.

THE
DAIRY LABORATORIES A DIVISION OF DALARE ASSOCIATES
23rd & Locust Sts., Phila., Pa.
BRANCHES
New York Baltimore Washington
See our catalog in Dairy Industries Catalog.

STANDARD "HANSEN'S" OF THE WORLD

Dairy Preparations

Cheese Rennet and Color
Annatto Butter Color
Certified Butter Color
Ice Cream Color
Lactic Ferment Culture
Bulgarian Culture

—also—

Cheese Bandages, Circles
Press Cloths
Odorless Dairy Fly Spray
Testing Solutions
Rennet Tests

Chr. Hansen's Laboratory, Inc.
Milwaukee, Wisconsin

Your advertisement is being read in every State and in 25 Foreign Countries

Our bulletin 30c is fully descriptive—Write for it.

The Gaulin Homogenizer is sanitary, easy to clean and approved by all leading health boards.

It is now available in 50 gallon to 2000 gallon per hour capacities and each is equipped with the Gaulin Two Stage Valve—a patented feature. The Gaulin is the ideal all purpose machine—used for processing Homogenized Milk, evaporated milk, ice cream and any other dairy product demanding a uniform fat dispersion with regulated viscosity.

MANTON-GAULIN TWO STAGE HOMOGENIZER

THE MANTON-GAULIN MFG. CO., INC.
7 CHARLTON STREET EVERETT, MASS., U.S.A.

International Association of Milk Sanitarians

will meet in

St. Louis, Missouri, October 30 and 31

Hotel Jefferson :: Official Headquarters

THE COMPOUNDING OF FINE VANILLA FLAVORING *IS AN ART*

The leadership of Mixevan for quality is the result of over 30 years specialized experience. Its uniform character is achieved through expert knowledge, individual selection of beans, extra development of the bouquet, intricate compounding and special grinding technique . . . *It is the ultimate in fine flavoring for dairy products.*

MICHAEL'S MIXEVAN
AMERICA'S FLAVORITE
MADE FROM BOURBON AND MEXICAN VANILLA BEAN, VANILLIN AND SUGAR
EXPERTLY BLENDED AND PROCESSED TO PRODUCE A DELIGHTFUL MELLOW MILD FLAVOR
DAVID MICHAEL & CO.
MANUFACTURERS
VANILLA PRODUCTS
FRONT AND MASTER STS.
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MARSCHALL RENNET

FOR PERFECT RESULTS IN CHEESE MAKING! AMERICA'S FAVORITE BRAND—IT IS ALWAYS STRONG, PURE AND UNIFORM.

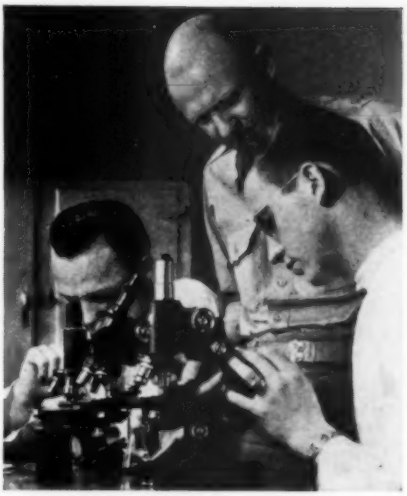
Marschall Dairy Laboratory
Incorporated
Madison, Wisconsin

GOOD NUTRITION
In Practice

Milk and its products contribute more to good nutrition than any other food group. Present consumption of all dairy products falls far below amounts recommended by scientific authorities.

Increased consumption of dairy products to attain these standards is the goal of the

NATIONAL DAIRY COUNCIL
111 North Canal St. Chicago, Ill.



*Spencer Microscopes in use in an Army Laboratory. Official Photo—Lowry Field**

The Greatest Health Crusade in History

Health is the first prerequisite of a soldier. Keeping him healthy is an essential ingredient of Victory.

Since World War I medical science has crusaded relentlessly for greater knowledge to insure better health. The success of this crusade is resulting in an army which is far healthier than any in history. Of equal importance is the fact that the nation behind our armed forces is likewise one of the healthiest which the world has ever known.

Today, as for over a century, Spencer Microscopes are playing an important role in both medical progress and applied medical practice. Greatly increased production facilities are in operation night and day to meet the unprecedented wartime needs for microscopes.

★ ★ ★

Optical instruments are so vital to war, production for war and the public health that the nation's needs absorb practically all of Spencer's greatly increased production.

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Spencer Lens Company
BUFFALO, NEW YORK
Scientific Instrument Division of
AMERICAN OPTICAL COMPANY

Sales Offices: New York, Chicago, San Francisco, Washington, Boston, Los Angeles, Dallas, Columbus, St. Louis, Philadelphia, Atlanta

** This, of course, does not constitute an indorsement by the War Department or by any other Government Agency.*

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"The last word in performance"

Model 541 Sanitary Viscolizer, capacity 500 gallons, in Engel Dairy, Philadelphia.

The Model 41 SANITARY VISCOLIZER

"still using the original packing leathers that come with the machine . . . a smooth, easy-to-clean apparatus . . . the last word in performance" . . . so writes Mr. Raymond W. Engel concerning his Model 541 Sanitary Viscolizer. In common with other Viscolizer users all over America, Mr. Engel has found the satisfying answer to his homogenizing problem . . . absolute and easily maintained sanitation, "the last word" in homogenization performance, complete dependability, bed-rock economy of operation and maintenance.

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If you have a homogenization problem take it up with your Cherry-Burrell representative. Ask him to get you a copy of illustrated Bulletin G-401 . . . or drop us a line.

CHERRY-BURRELL CORPORATION

427 WEST RANDOLPH STREET, CHICAGO

and

FACTORIES, WAREHOUSES, BRANCHES, OFFICES OR DISTRIBUTORS AT YOUR SERVICE IN 55 CITIES

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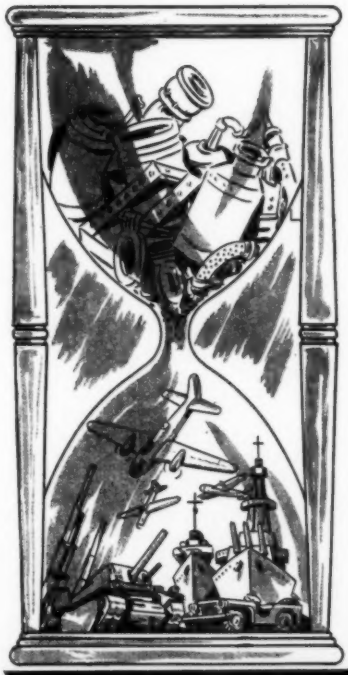
SCRAP NOW

For Victory Later!

"*The Philosophy of* 'It May Come in Handy Some Day' Must Give Way to the Doctrine of '*My Country Needs It Now!*'"



National Dairy Industry Salvage Drive
Sponsored by
War Production Board
Salvage Branch of Conservation Div.

D. M. NELSON, Chairman
War Production Board

Dairy products plants—large and small—have been called upon for aid in a very vital phase of the war effort. The War Production Board is counting on our industry as one of the best sources of copper, brass, bronze, tin and other non-ferrous metals. It is also asking us to help build up the Nation's stock pile of stainless steel, iron, steel and rubber.

The job is more than simply cleaning out odds and ends that were discarded months or years ago. It's a job that sometimes takes courage. It means measuring machinery and fixtures that we've figured might "come in handy some day" with a wartime yardstick . . . and condemning every part we can possibly do without. Maybe you have saved a complete machine because it included one or more usable parts. By all means save those parts, but get the unusable material in the scrap.

The first thing to do is to put some one individual in charge of Salvage . . . and give him authority to act. Then sell or donate your accumulation of scrap and condemned equipment. Be sure to get a receipt—broken down by types of metal. It is important that we have records showing the volume and kinds of scrap salvaged from the Dairy Industry.

Consult your equipment or supply house representative for important details on getting your scrap in the scrap at once . . . and getting it credited to your industry.

This advertisement contributed by

THE CREAMERY PACKAGE MFG. COMPANY

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DIFCO

CULTURE MEDIA

for Examination of Milk

Bacto-Tryptone Glucose Extract Agar
is recommended for use in determining the total bacterial plate count of milk in accordance with the procedures of "Standard Methods for the Examination of Dairy Products" of the American Public Health Association.

Upon plates of medium prepared from Bacto-Tryptone Glucose Extract Agar colonies of the bacteria occuring in milk are larger and more representative than those on media previously used for milk counts.

Bacto-Proteose Tryptone Agar
is recommended for use in determining the bacterial plate count of Certified Milk. The formula for this medium corresponds with that suggested in "Methods and Standards of Certified Milk" of the American Association of Medical Milk Commissions.

Bacto-Violet Red Bile Agar
is widely used for direct plate counts of coliform bacteria. Upon plates of this medium accurate counts of these organisms are readily obtained.

Bacto-Brilliant Green Bile 2% and Bacto-Formate Ricinoleate Broth
are very useful liquid media for detection of coliform bacteria in milk. Use of these media is approved in "Standard Methods."

Specify "DIFCO"
THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES
INCORPORATED
DETROIT, MICHIGAN

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